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MATHEMATICAL SIMULATION OF DEFORMATION FRICTION FORCE DURING FOOD MATERIAL CUTTING

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ABSTRACT

The process of friction when cutting food materials has been investigated theoretically. The muscle tissue of the raw material has been described by the Maxwell-Thomson rheological model. When choosing an analytical description of a regular microrelief of food processing equipment knives, taking into account technological formative factors, a physical-technological theory of surface roughness has been used. A mathematical description of the profile of the knife rough surface in the form of a dimensionless periodic one-parameter function has been obtained. By solving the differential equation of viscoelastic material state in a dimensionless form, the law of distribution of dimensionless normal contact pressures over the microprotrusions of the edge rough surface has been obtained. An expression for the dimensionless deformation friction force has been obtained. It is established that at speeds tending to zero or infinity, this force tends to zero. The magnitude of the force increases monotonically with the increase in the measure of the material elasticity and the increase in the dimensionless length of the knife edge. The dimensionless width of the contact area of microprotrusion is monotonously depends on the measure of material elasticity and non-monotonously depends on the dimensionless sliding speed with a pronounced minimum.

1.Introduction

Ensuring resource saving when cutting food provides a thorough analysis of the resistance forces. Reducing the strength of harmful resistance involves minimizing its important component - the deformation force of friction.

The friction force of the food material and the knife surface is a consequence of the edges roughness, which is determined by the parameters of the technological processing of the working body (Grigoriev, 2016). The advanced technology for the production of knives for food processing equipment includes the following main types of operations: machining (finishing turning) and grinding processing, as a result of which the surface profile includes random and systematic components.

Mathematical modeling and experimental study of the friction process of food materials on a rough surface is a current scientific direction in engineering. Spagnoli et al. (2019) have investigated the effect of friction and the blade immersion depth on the resistance force when cutting viscoelastic materials. Also Spagnoli et

al. (2018) have analyzed the laws of the process of destruction of a viscoelastic material with friction, have analyzed the effect of sharpness of the cutting unit on the distribution of stresses in the material.

Xiao-Ping Zhou et al. (2019) have performed an experimental theoretical analysis of the mechanical behavior of highly elastic media in the process of deformation and fracture. Schuldt et al. (2018) have examined the laws of friction in the food materials processing in a wide range of speeds: from 0.001 m/s to 10 m/s.

Belaasilia et al. (2017; 2018) have described numerical modeling of contact phenomena at the separation surface of elastic and rigid bodies. Wang et al. (2016) and Jadav et al. (2018) have investigated the laws of the friction process of various materials when changing the slip mode. Deibel et al. (2014) have analyzed the patterns of cutting multilayer media and have shown the effect of friction forces on the destruction of the material. Kieserling et al. (2018), Winkeljann et al. (2018) have developed modern methods and installations for measuring the tribological characteristics of food products.

However, despite the value of the known works, there is currently no analytical description of the friction forces acting on the working body when cutting food materials. At the same time, to optimize the geometry of the knife according to the criterion of minimum cutting resistance, mathematical modeling of the forces acting on its edges, taking into account the surface roughness, is required.

2.Materials and methods

Ageev et al. (2018) have substantiated the choice of rheological models of tissue of viscoelastic food products of animal origin. Differential equations of models with their solutions for three different loading conditions have been considered. It has been established that the fish muscle tissue, until destruction, exhibits a limited flow under load, relaxes under constant load to an equilibrium state, and is fully restored when fully unloaded. It is shown that the results of the experimental testing of the material at low and medium voltages approximately correspond to the three-element Maxwell – Thomson rheological model. (standard viscoelastic body).

2.1.Mathematical simulation

When choosing an analytical description of a regular microrelief of the working parts of food processing equipment taking into account technological formative factors, the physical-technological theory of surface irregularities has been used (Grigoriev, 2016). The effect of the formative factors in finishing turning is periodic or practically periodic in nature, due to the tool feed, the workpiece turn, the abrasive tool self-sharpening and other conditions. If we assume that the random component is small, then the basis of the microrelief of the knife edge surface is approximately described by a trigonometric polynomial of the form:

$$f(x)=\frac{a_0}{2}+\sum_{n=1}^{p}\left\{a_n\cdot\cos\left(\frac{2\pi\cdot n\cdot x}{S_0}+\overline{\psi}_n\right)\right\}, \quad (1)$$

where S_0 – the step of the profile (step of the first harmonic); p – the order of the polynomial (number of harmonics); a_n, $\overline{\psi}_n$ – the Fourier coefficient and phase angle of the n-ith component of the profile; $a_0/2$ – the zero term of the expansion for the profile curve (coordinate of the center line of the profile in such a coordinate system in which the ordinate is parallel to the center line of the profile).

Denote that $a_0 = A$; $a_1 = 0.5\cdot A$.

A model of a periodic surface profile containing one harmonic is expressed from (1) in the following form:

$$f(x)=A\cdot\cos^2\left(\frac{\pi\cdot x}{S_0}+\overline{\psi}\right). \quad (2)$$

Differential equation of the material state:

$$\frac{dp}{dx}u+p\frac{E_0+E_1}{\eta}=\frac{df}{dx}uE_0+f\frac{E_0\cdot E_1}{\eta}, \quad (3)$$

where p – contact pressure; E_0 – instantaneous modulus of material elasticity (Young's modulus); E_1 – delayed modulus of

material elasticity; η – coefficient of dynamic viscosity of material; u – knife speed.

The solution of the differential equation (3) with the initial condition $p(0)=0$ is:

$$p(x)=\frac{A\cdot\xi\cdot k}{A_0\cdot\left(4\pi^2+k^2\cdot S_0^2\right)}\cdot\left\{-\left(\vartheta+0.5\cdot k\cdot S_0^2\right)\cdot\cos\left(\frac{2\pi\cdot x}{S_0}\right)-\right.$$

$$\left.-\pi\cdot e_{01}\cdot S_0\cdot\sin\left(\frac{2\pi\cdot x}{S_0}\right)+0.5\cdot k\cdot S_0^2+\frac{2\pi^2}{k}+\left[\vartheta-\frac{2\pi^2}{k}\right]\cdot\exp(k\cdot x)\right\}, \quad (4)$$

where $k=-\frac{E_0+E_1}{\eta\cdot u}$; $\xi=\frac{E_0\cdot E_1}{E_0+E_1}$; $\vartheta=\frac{2\cdot\pi^2\cdot E_0}{\xi\cdot k}=\frac{2\cdot\pi^2}{k}\cdot(e_{01}+1)$; $e_{01}=\frac{E_0}{E_1}=\left(\frac{E_0}{\xi}-1\right)$.

$$F_2=l_k\cdot\int_0^{x_B}\left(\frac{df}{dx}\cdot p(x)\right)dx=l_k\cdot\int_0^{x_B}\left\{\frac{\pi\cdot A}{S_0}\cdot\sin\left(\frac{2\pi\cdot x}{S_0}\right)\cdot p(x)\right\}dx=$$

$$=\frac{\pi\cdot A^2\cdot\xi\cdot k\cdot l_k}{A_0\cdot S_0\cdot\left(4\pi^2+k^2\cdot S_0^2\right)}\cdot\int_0^{x_B}\left\{\sin\left(\frac{2\pi\cdot x}{S_0}\right)\cdot\left[-\left(\vartheta+0.5\cdot k\cdot S_0^2\right)\cdot\cos\left(\frac{2\pi\cdot x}{S_0}\right)-\right.\right.$$

$$\left.\left.-\pi\cdot e_{01}\cdot S_0\cdot\sin\left(\frac{2\pi\cdot x}{S_0}\right)+0.5\cdot k\cdot S_0^2+\frac{2\pi^2}{k}+\left(\vartheta-\frac{2\pi^2}{k}\right)\cdot\exp(k\cdot x)\right]\right\}dx. \quad (5)$$

Lets use the Newton-Leibniz formula and obtain from (5) an expression for the deformation friction force:

$$F_2=\frac{\pi\cdot A^2\cdot\xi\cdot k\cdot l_k}{A_0}\cdot J_1\cdot\left\{\frac{1}{8\pi}\cdot\left[I_1\cdot\cos\left(\frac{4\pi\cdot x_B}{S_0}\right)+\pi\cdot e_{01}\cdot S_0\cdot\sin\left(\frac{4\pi\cdot x_B}{S_0}\right)\right]+\right.$$

$$+J_1\cdot k\cdot S_0\cdot\left(\vartheta-\frac{2\pi^2}{k}\right)\cdot\sin\left(\frac{2\pi\cdot x_B}{S_0}\right)\cdot\exp(k\cdot x_B)-$$

$$-\frac{J_1}{2\pi}\cdot\cos\left(\frac{2\pi\cdot x_B}{S_0}\right)\cdot\left[\left(0.5\cdot k\cdot S_0^2+\frac{2\pi^2}{k}\right)\cdot\frac{1}{J_1}+4\pi^2\cdot\left(\vartheta-\frac{2\pi^2}{k}\right)\cdot\exp(k\cdot x_B)\right]+$$

$$\left.+\frac{J_1}{2\pi}\cdot\left[\left(0.5\cdot k\cdot S_0^2+\frac{2\pi^2}{k}\right)\cdot\frac{1}{J_1}+4\pi^2\cdot\left(\vartheta-\frac{2\pi^2}{k}\right)\right]-0.5\cdot\pi\cdot e_{01}\cdot x_B-\frac{I_1}{8\pi}\right\}, \quad (6)$$

where $J_1=\frac{1}{\left(4\pi^2+k^2\cdot S_0^2\right)}$; $I_1=\vartheta+0{,}5\cdot k\cdot S_0^2$.

Lets introduce the dimensionless velocity $\bar{u}$ and dimensionless coordinate $\bar{x}$:

$$u = \bar{u} \cdot \frac{S_0 \cdot (E_0 + E_1)}{\eta}; \qquad k = -\frac{1}{\bar{u} \cdot S_0}; \qquad J_1 = \frac{1}{4\pi^2 + k^2 \cdot S_0^2} = \frac{\bar{u}^2}{4\pi^2 \cdot \bar{u}^2 + 1};$$

$I_1 = -S_0 \cdot \left(\frac{0.5}{\bar{u}} + 2\pi^2 \cdot \bar{u} \cdot (e_{01} + 1) \right)$; $\frac{x_B}{S_0} = \bar{x}_B$; $k \cdot x = -\frac{\bar{x}}{\bar{u}}$. Taking into account $F_0 = \left(A^2 \cdot \xi \cdot l_k \right) / A_0$;

$F_2 = \bar{F}_2 \cdot F_0$ we obtain the expression for the dimensionless deformation force of friction:

$$\bar{F}_2 = \frac{J_1}{\bar{u}} \cdot \left\{ -0.125 \cdot \left[\bar{I}_1 \cdot \cos(4\pi \cdot \bar{x}_B) + \pi \cdot e_{01} \cdot \sin(4\pi \cdot \bar{x}_B) \right] - \right.$$
$$- 2\pi^3 \cdot J_1 \cdot e_{01} \cdot \sin(2\pi \cdot \bar{x}_B) \cdot \exp(-\bar{x}_B / \bar{u}) -$$
$$- 0.5 \cdot \cos(2\pi \cdot \bar{x}_B) \cdot \left[\frac{0.5}{\bar{u}} + 2\pi^2 \cdot \bar{u} + 8\pi^4 \cdot J_1 \cdot \bar{u} \cdot e_{01} \cdot \exp(-\bar{x}_B / \bar{u}) \right] +$$
$$\left. + 0.5 \cdot \left[\frac{0.5}{\bar{u}} + 2\pi^2 \cdot \bar{u} + 8\pi^4 \cdot J_1 \cdot \bar{u} \cdot e_{01} \right] + 0.5 \cdot \pi^2 \cdot e_{01} \cdot \bar{x}_B + 0.125 \cdot \bar{I}_1 \right\}. \qquad (7)$$

3. Results and discussions

Fig. 1 shows the dependence of the dimensionless deformation friction force along the entire knife edge on the dimensionless sliding speed by various dimensionless length of the edge and measure of the material elasticity. Fig. 2 shows the dependence of the dimensionless deformation friction force along the entire knife edge on the dimensionless length of the edge by different sliding speed and measure of elasticity. The contour graph fig. 3 shows the dependence of the dimensionless deformation friction force on the dimensionless length of the edge and the dimensionless sliding speed, the contour plot fig. 4 - from the dimensionless speed and measure of the material elasticity.

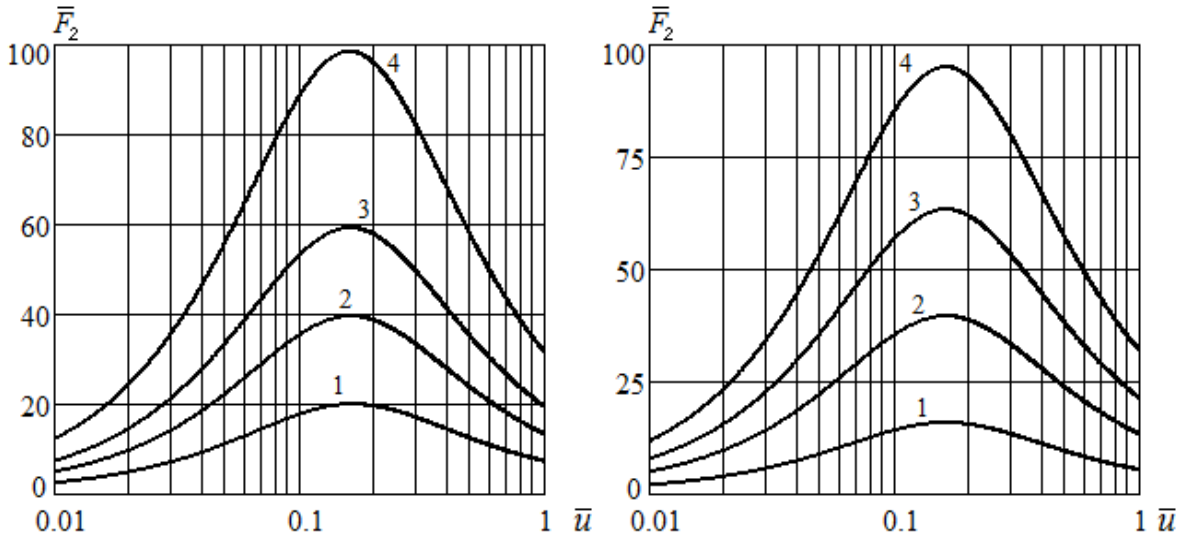
Calculations of the dimensionless deformation friction force on the fig. 1-4 are drawn in accordance with the dimensionless length of the edge $\bar{x}_B$. Analysis of expressions (6), (7) shows that the dimensional deformation friction force depends on the height of the microprotrusions, the height of the deformable surface layer of the material, the length of the knife edge, the rheological properties of muscle tissue, the speed of sliding and the edge length of the knife.

Fig. 1 demonstrates that the dimensionless deformation friction force is a non-monotonic function of the dimensionless sliding speed. At slip speeds tending to zero or infinity, this force tends to zero. Zero force corresponds to the solution of the problem for indentation of microprotrusions into elastic material, which is characterized by a long modulus of elasticity ξ. The magnitude of the friction force increases significantly with increasing material elasticity.

Fig. 2 shows that the deformation force of friction increases significantly with an increase in the dimensionless length of the knife edge. As fig. 3 illustrates the dependence of the indicated friction force on the length of the edge is monotonous, as well as the dependence on the measure of the material elasticity according to fig. 4.

With the values of the measure of elasticity 5, dimensionless edge length 10; 20; 30; 50, values of the maximum deformation force of friction are 19.958; 39.585; 59.220; 98.489,

respectively. At the values of dimensionless face length 20; elasticity measures 2; 5; 8; 12, values of the maximum deformation force of friction are 15.834; 39.585; 63.336; 95.004, respectively.



a *b*

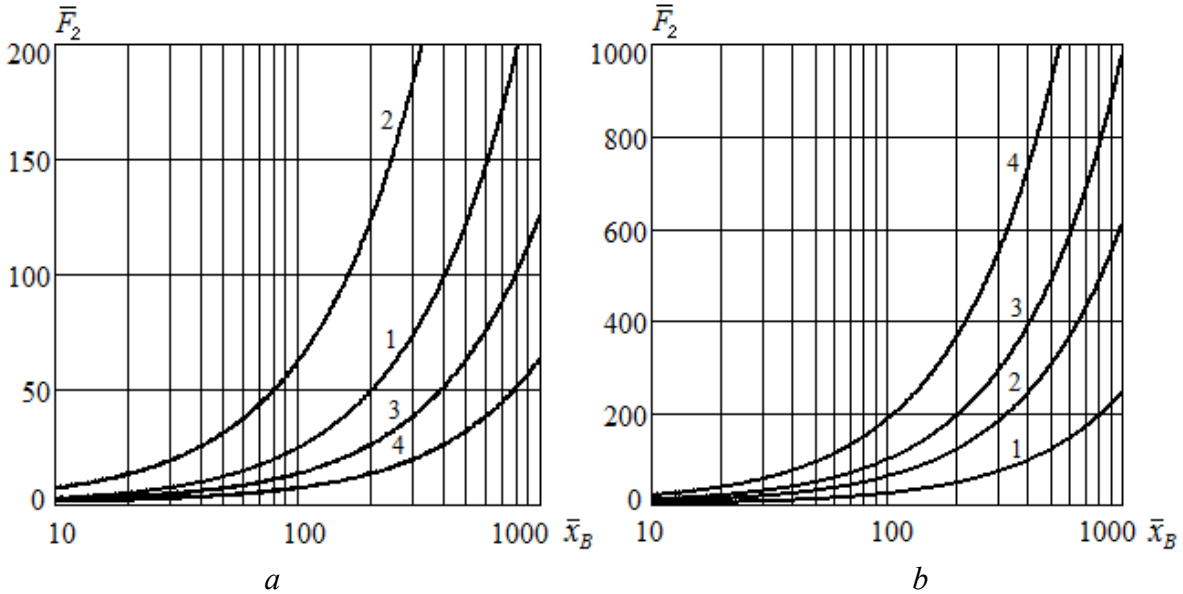
Figure 1. The dependence of the dimensionless friction force on the dimensionless sliding speed (scale $\bar{u}$ - logarithmic):

a –at various values of the dimensionless length of the edge ($e_{01} = 5$):

$1 - \bar{x}_B = 10; 2 - \bar{x}_B = 20; 3 - \bar{x}_B = 30; 4 - \bar{x}_B = 50;$

b – at different values of the measure of material elasticity ($\bar{x}_B = 20$):

$1 - e_{01} = 2; 2 - e_{01} = 5; 3 - e_{01} = 8; 4 - e_{01} = 12$



a *b*

Figure 2. The dependence of the dimensionless friction force on the dimensionless length of the edge (scale $\bar{x}_B$ – logarithmic):

a – at various values of the dimensionless sliding speed ($e_{01} = 5$):

$1 - \bar{u} = 0{,}01$; $2 - \bar{u} = 1$; $3 - \bar{u} = 5$; $4 - \bar{u} = 10$;

b – at different values of the measure of material elasticity ($\bar{u} = 1$):

$1 - e_{01} = 2$; $2 - e_{01} = 5$; $3 - e_{01} = 8$; $4 - e_{01} = 15$

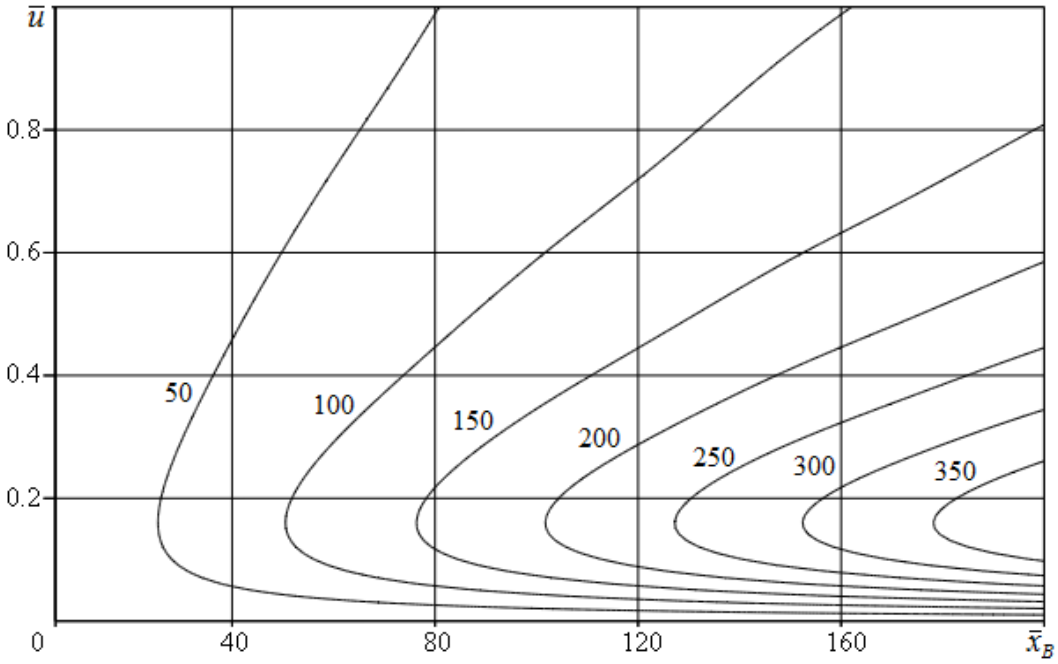


Figure 3. The dependence of the dimensionless friction force $\bar{F}_2$ on the dimensionless length of the edge and on the dimensionless sliding speed ($e_{01} = 5$)

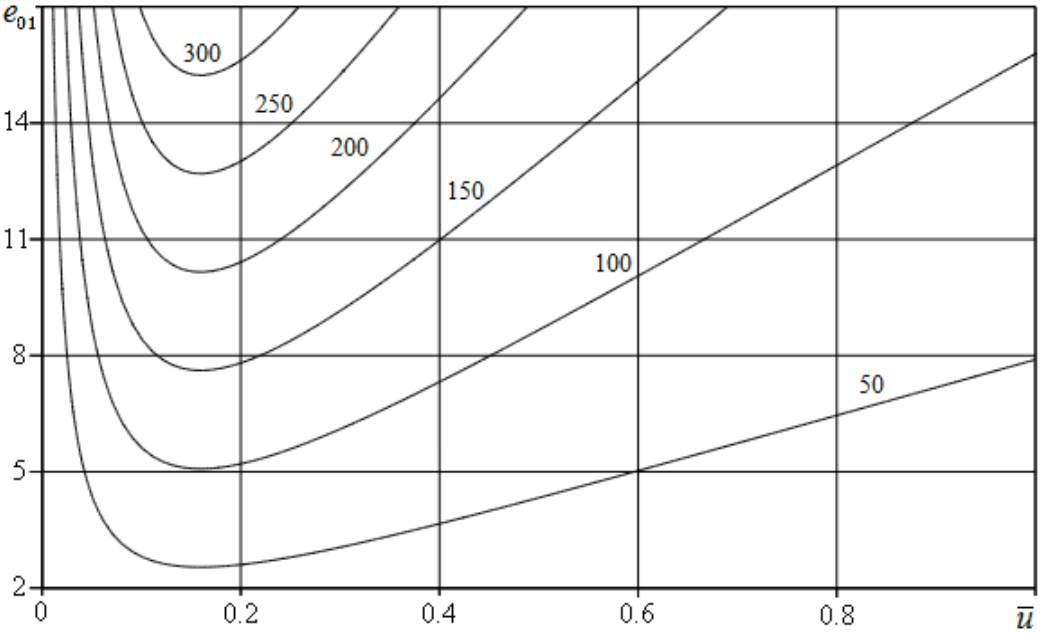


Figure 4. The dependence of the dimensionless friction force $\bar{F}_2$ on the dimensionless sliding speed and on the measure of material elasticity ($\bar{x}_B = 50$)

5.Conclusions

The distribution of contact pressures and the deformation friction force of the knife substantially depend on the roughness shape of the rough surface of the edge, as well as on the sliding speed and the rheological properties of the food material. When a certain value of speed is reached, this force has a pronounced maximum, after which it nonlinearly decreases.

The dimensionless width of the contact area of the microprotrusions x_c monotonously depends on the measure of the material elasticity and non-monotonously depends on the dimensionless sliding speed with a pronounced minimum.

The established dependences make it possible to scientifically manage the geometric shape of the unevenness of the technological roughness of a knife in order to minimize the deformation friction force when cutting food materials.

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PROCESS AND PRODUCT OPTIMIZATION IN THE ARTISANAL PRODUCTION OF SICILIAN COFFE GELATO

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ABSTRACT

The production of artisan gelato has always been a very important sector for the Italian economy, the aim of this work is to highlight the peculiarity of production in Sicily, in synergy with a young economic reality and the production reality that is expanding Gelateria Cappadonia, Caffè Morettino Srl Palermo and the academic world.

The aim of this research is to solve the color change during the production of artisan coffee gelato.

1.Introduction

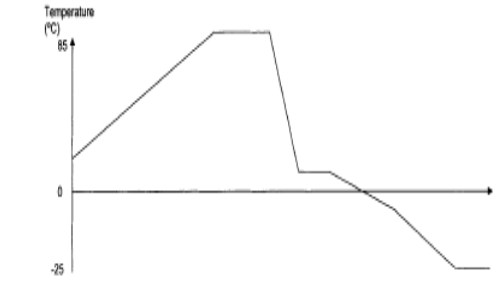
The artisanal production of gelato is widespread in Italy and in Sicily some companies represent an excellence in the sector. The production methods of these companies, in most cases have changed a lot over time, being able to take advantage of a technological development that is present in the sector, which has safe and reproducible food products for its organoleptic characteristics. This prompted the work of the gelato masters for the development of new recipes and formulas of gelato and various sorbets. In fact, production is not always essential due to problems or requires some form of optimization (process or product) to better meet market needs. In these cases, the approach to solving problems is often empirical, almost always based on the experience of the master gelato maker. The activity carried out in this experimental work stems from the need to find a solution to a problem of "coloring" of a type of gelato with an approach that was not simply empirical but also made use of a scientific basis. Gelato is a complex food which in the frozen state consists of ice crystals, air and fat droplets dispersed in an aqueous phase (called serum) containing polysaccharides such as galactomannans, carrageenan (food gelatin), cellulosics, sugars (sucrose and lactose) , proteins and minerals (especially calcium, but also sodium and potassium) (Soukoulis *et al*., 2014). The term "gelato" includes a wide range of frozen desserts (Clarke, 2005) and its composition varies depending on the country and the demand of the country's consumer. The main families are: creams, sorbets and granitas. The creams are in turn divided into egg creams and milk creams, depending on the presence or absence of the first ingredient that has the emulsifying function. For this type of gelato, the pasteurization process is necessary in order to

remove the bacterial load present. The white cream family includes, among others, vanilla, cinnamon and coffee gelato.The parameters established for a cream gelato set the amount of water around 64% of the total, while the rest, 36%, is reserved for the dry extract, always providing a margin of variation determined by the characteristics of each gelato (Corvitto, 2005). The sorbet means a frozen product based on water, sugars and neutral stabilizer. A cause of the lack of raw materials and milk powder, sorbets retain all the genuine flavor of the fruit or raw material used and for the reason given and refreshing. Generally, the sugar content in sorbets is twice that of gelato. Compared to creams, sorbets have a higher fruit content and a thicker consistency which therefore causes a greater feeling of cold on the palate (Douglas, Hartel, 2013). The peculiarity of this type of product is the unnecessary pasteurization. This is because almost all fruit has a pH <4.6, which indicates the absence of bacterial load means pathogenic, as it does not survive in such conditions. For sorbets the amount of water is about 70%, while the dry amount is about 30%. Granitas represent ice water containing sugar and flavors. It is similar to the sorbet, with the difference in communication in this case there is the complete absence of neutral. Another difference lies in the slow agitation which, for the reason, causes a rougher consistency caused by the larger ice crystals. (Douglas, Hartel, 2013). The ice crystals must be very fine, noticeable on the tongue and easily melt in the mouth. Generally the percentage of sugar is between 14 and 24%. The sugar most used in the preparation of granite is sucrose. The aim of this research is to solve the color change during the production of artisan coffee gelato.

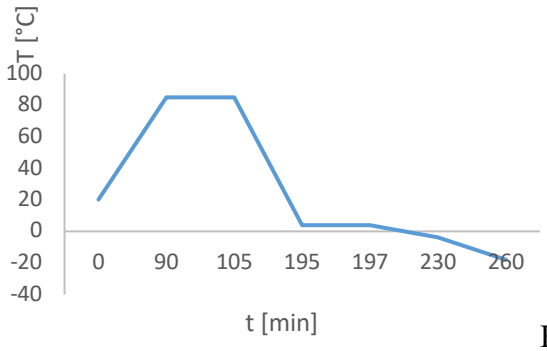
2. Materials and methods

The experimental tests were carried out at the Cappadonia Company's artisan factory in Cerda, in the province of Palermo. The main functions of the sugar inside the gelato are to give sweetness, increase the viscosity of the final product (thus improving the structure) and lower the freezing point. The higher the sugar content, therefore, the lower the ice content, therefore the softer the gelato will be. Neutrals, represented by emulsifiers and stabilizers, have a very important role for the final structure and for the quality of the gelato. The goal for the production of a high quality gelato is that the water and fats are eliminated in such a way as to prevent the two phases from returning to two separate phases. The liquid phase in gelato is largely composed of water, its quantity is about 60%. It plays a fundamental role for the production of gelato, without it, in fact it would not be possible. The gas phase is represented by air. The air incorporated in the gelato causes it to be lighter, has a lower specific heat capacity (both felt in the mouth as less "cold") and is also creamier. The increase in the volume of an gelato mix, defined by the incorporated air, is called overrun (Corvitto, 2005). A method for determining the optimal amount of air for gelato is described by the following equation 1:

$$overrun = \frac{density\ mix - density\ ice\ cream}{density\ ice\ cream} x100 \quad (1)$$



A



B

Figure 1. A-B (A) Temperature profile during the gelato production process (Clarke, 2004) and

(B) temperature profile during the experimental gelato production process.

The production process involves a precise weighing, before the gelato production process. This occurs through the succession of different phases: mixing, pasteurization and homogenization, maturation, freezing, temperature reduction, conservation and finally exposure. Due to these multiple steps, the mixture follows a variable temperature profile. In fact, it first grows from room temperature up to 85 °C, during the pasteurization process, then decreases down to -25 °C in the following steps (Fig. 1).

It is important to highlight the process of reducing the temperature since during the process the process of crystallization of the water takes place. It occurs in four stages: lowering the temperature, nucleation, growth and recrystallization. Crystallization of the solvent occurs when the temperature drops below the freezing temperature of the mixture. As we know, the freezing temperature of the water is 0 °C, without the presence of further scarce substances that lower its cryogenic point. The size of the ice crystals also lowers the temperature at which crystals of different sizes crystallize due to the increase in free energy necessary to overcome surface tensions when a new interfacial surface is created. However, the formation of new ice crystals starting from the liquid phase implies the creation of a solid-liquid interfacial surface which requires an extra energy compared to the thermodynamic equilibrium. What corresponds to the temperature at which it is represented in the new crystals (in the absence of any solid phase, including impurities) is much lower than that of equilibrium the smaller the crystal. This phenomenon, explained by equation 2, depends on free energy (on surface tension); since small crystals have more free energy than larger crystals, their freezing point is lower (Cook, Hartel, 2010).

$$\Delta T_c = \frac{2\sigma T_\infty}{\rho_s \Delta H_{fus} r} \quad (2)$$

In which:

σ = surface tension

ΔHfus = latent heat of fusion

ρ = density of the ice crystal

r = critical radius of the crystal

Nucleation described the birth of the crystal (Hartel, 2001). The nucleation of the ice crystals takes place starting from the freezing phase. The very small nuclei found on the internal walls of the batch freezer are scraped by the moving blades (or screws) and involve the site of the growth of the ice crystals.
The distribution of the typical size of the ice crystals inside the gelato is shown in Fig. 2.

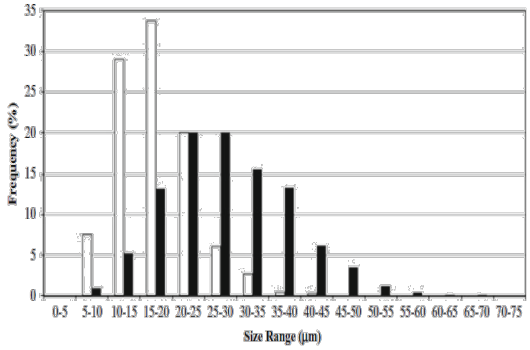


Figure 2. Frequency of distribution of ice crystals in gelato (Douglas, Hartel, 2013)

As can be seen from the graph, the ice crystals vary in size. The greatest distribution occurs between 10 and 25 µm (immediately after the freezing phase, in white) and between 25 and 40 µm (after the temperature has been lowered, in black) (Douglas, Hartel 2013). After stable nuclei have formed they grow to form crystals. A fundamental ingredient of gelato is coffee, of which there are about 80 types of varieties (Grassia et al., 2019). The most common commercial varieties are only two: *Coffea Arabica* and *Coffea Robusta*. The quantity of these components inside the beans is variable and depends mainly on genetic aspects, the maturation to which they are subjected and the processing techniques (Vecchio et al., 2012).

The chemical composition of green beans is made up of water, carbohydrates, fibers, proteins, amino acids, lipids, minerals, organic acids and caffeine (Tab. 1).

Table 1. Chemical composition of coffee (Chu, 2012)

Carbohydrates and fibers	
Components	**Coffee'(g/100g)**
Sugar	6-9
Polysaccharides	34-44
Lignin	3
Pectin	2
Nitrogen compounds	
Components	**Coffee'(g/100g)**
Protein	10-11
Caffeine	0.9-1.3
Lipid	18.55-22.2
Mineral	5.5-9.3
Water	4.1

Carbohydrates are precursors of Maillard's reactions. This reaction occurs during the roasting of green coffee beans. During this process, in fact, sugars combine with amino acids and represent a high number of high molecular weight components responsible for the color variation of coffee and some antioxidant properties (Thurston *et al*., 2013). The plot, created through sensory analysis.

3.Results and discussions

The first test was that of the artisan production of granita with the ice method and the sale with the instrument of the quantity of sale, the production of cold is more intense. Once the mixture was introduced into the carapine, the temperature was monitored, considering this as an important initial point. Temperature monitoring was detected in the ice, in the volume (internal surface of the carapina) and in the heart (center of the mixture) of the carapina attested by photographic image. Initially the mixture was rather dark as can be seen from Fig. 3.

Figure 3. Mixture just introduced inside the carapina

After 35 minutes, the consistency and coloration of the roasted coffee is noted, as can be seen in Fig. 4.

Figure 4. Coloring of the granita after 35 minutes

The texture, created by sensory analysis, the finish of the granita is excellent on the palate and very soft. As can be seen from Fig. 5 the resulting color is black, typical of freshly roasted coffee. The goal would be to report this result also in the production of sorbet, gelato and granita with an industrial machine.

Figure 5. Coloring of the granita after 35 minutes

In the following graph (Fig. 6) instead it is possible to notice the trend of the three temperatures with time.

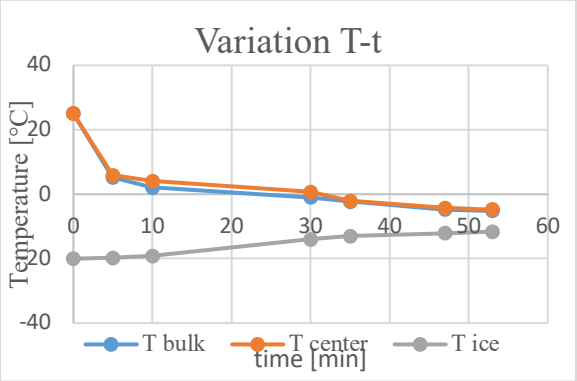


Figure 6. Variation of temperatures over time

The production of artisanal gelato with a horizontal batch freezer begins with the extraction of the coffee and the relative one with high pasteurization with cream. This step is necessary to reduce the bacterial load, but also to favor the melting of sugars and denature the proteins. Parallel to pasteurization, the homogenization of the phase in which the components are emulsified through the crushing of the fat globules also takes place. The output of the pasteurizer can be seen as the coffee-cream-powder mixture has lost color. This is quite obvious due to the presence of the cream which acts as a "whitener" (Fig.7).

Figure 7. Cream-coffee-sugar mixture after pasteurization

Figure 8. Representation of coffee gelato

At the exit of the batch freezer the coffee cream is clear (Fig. 8). The number of revolutions of the auger and of the feed pump has been changed. Note how the color is unchanged from before. The only change reported is to the texture of the gelato, as this is more "wet" and less delicate on the palate. The production of artisanal sorbet with a horizontal coffee batch freezer takes place, once the coffee is extracted, sugars and neutrals are added and mixed in order to obtain a homogeneous mixture. The final mixture will have the following composition by weight: 59% coffee, 39.7% sugars and fibers (inulin, chicory root extract), 0.3% neutral. At the exit of the batch freezer the texture is comparable to that of the coffee cream, although it is only a mixture containing coffee, sugars and neutrals. In Fig. 9 it is possible to notice the difference in color of the creamed coffee sorbet and the mixture of non-creamed liquid coffee.

Figure 9. Color difference of the liquid mixture and the sorbet

(a) (b) (c)

Figure 10. Coffee sorbets with the addition of micronized coffee 30 µm (a), 50 µm (b), 100 µm (c)

(a) (b) (c)

Figure 11. Coffee cream with the addition of micronized coffee 30 µm (a), 50 µm (b), 100 µm (c)

Following the results obtained previously in the production of coffee granita, coffee milk cream and coffee sorbet, it is clear that the problem posed by the Cappadonia company actually arises. To be able to remedy this problem, it was thought to be able to add the micronized coffee inside the pre-certified mixture. For this reason, thanks to the collaboration with Morettino Caffè, in Palermo, it has been possible to obtain ground coffee with three different millings in order to carry out three distinct tests (30 µm, 50 µm "Turkish coffee milling", 100 µm). Once the micronized coffee blend from Morettino is obtained, it is a procedure with the test of adding it to the coffee sorbet blends and pasteurized coffee creams, before the batch freezing process. However, no particular differences have emerged from the addition of micronized coffee in the coffee sorbet (Fig. 10) and gelato (Fig. 11).

Figure 12. Production of coffee granita with granita machine

Following the tests carried out, the color variation in the production of granita was monitored with the slush machine. The production of the granita had previously been done with the hand-made ice and salt technique. Remembering the above test, the color that came out was quite dark tending to black. We would therefore expect that the production of the same granita, starting from the same matrix, will be the same by varying the means of production. By re-proposing the low speeds in the granite machine, note that the result is similar to that obtained with the ice and sale technique (Fig. 12).

Figure 13. Texture of the granita as the number of turns increases

By increasing the number of revolutions, reaching 80 rpm, the color of the granita changes to a hazelnut color (almost caramel color) (Fig. 13)

From the tests carried out, it is therefore deduced that the color is modified as the stirring speed changes, resulting increasingly clearer with increasing speed. at low speeds from low temperatures, while at higher speeds from high temperatures. This observation was confirmed by increasing the stirring speed. For this reason, it was decided to re-propose the high speeds in the production of granita with the ice and salt technique. The aim was to verify that the color change was not caused by a chemical phenomenon but by a physical phenomenon or correlated with the increase in speed. The final color, after 3 minutes it was hazel colored as you can see in Fig. 14. From this it can be seen how the monument to speed adversely affects the coloring of the final product.

Figure 14. Color variation with increasing speed

The last test carried out on the coffee sorbet consisted in the production of it with the technique with ice and salt, at low and high speeds. It was expected, also for this test, that low speeds would improve the finished product, while high speeds would negatively affect the quality and coloring of the product. Indeed, this is what has been created. In this case too, in fact, at low stirring speeds (Fig. 15) it can be seen that the coloring is rather dark.

Figure 15. Color variation of the artisan sorbet

Increasing the speed you notice how already after a couple of minutes the color has both visibly changed. In fact, it is of a color similar to hazelnut (Fig. 16).

Figure 16.Coloring of the sorbet at high speeds

After 30 minutes from the end of the test, a change is noticed. The color of the hazelnut sorbet seemed to take on the original color again, that is, that of coffee (Fig. 17). This result shows that at low speeds, both for the granita and for the sorbet, the ice crystals, growing on a few nuclei, form large ice crystals reflecting the color of the mixture. The high speeds, for both preparations, instead break the large crystals and therefore they can no longer act as mirrors, effectively making the preparations lose their color. As you can see from the figure, the "hand" production also negatively affects the quantity of air incorporated by the product, the quantity of foam on the surface of the mixture is in fact quite high.

Figure 17. Melting of ice crystals and return to the original color

4. Conclusions

The production problem was to find a solution to the color variation in the sorbet and coffee granita. From the outset, the manifested problem apparently had the appearance of a chemical problem of a "chemical" nature. For this reason, the case study began with the search for which coffee molecule was negatively affecting the coloring of the finished product. Being both cold products, we paid attention to the ice crystals that were inevitably created during their production. At low speeds the ice crystals grew on a few nuclei creating rather large crystals, at high speeds, however, the crystals did not have time to grow on many nuclei, thus resulting rather small and invisible to the naked eye. The larger crystals, being clearly visible, reflected the dark color of the coffee, making both products appear "black". The smaller crystals, on the other hand, being invisible to the naked eye, did not reflect the color of the coffee, making the granita and sorbet appear "clear". This was demonstrated by melting products where high speeds had changed their color. Once the ice crystals were melted, the color turned dark again, confirming our results.

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STUDY OF SOLAR BASED VERTICAL FARMING SYSTEMS WITH RAY-TRACED DAYLIGHTING ANALYSIS AND VISUALIZATIONS

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ABSTRACT

This article explores the use of raytracing software and heat load models to optimise the growing of crops vertically and by doing so, serves to increase the plausibility of urban Vertical Farms (VFs). Crop trays are modelled using a computer aided design (CAD) software and is then imported into a raytracing software TracePro-2019. Parameters obtained from the heat load and angle calculations would be used to start the simulation. Thereafter, solar irradiance obtained would be compared to different simulations with different design parameters. For this paper, the main design variable would be the tray width and tray inclination angle. After multiple simulations, it was found that trays at the bottom are less affected by varying angles of sunlight while trays at the top receive extreme variations. It was also noted that while the shaded area increases as tray inclination increases, the solar irradiance flux on each tray increases as opposed to decreasing. This new finding led to the conclusion that an increase in the angle of attack of the solar irradiance increased the occurrence of reflected and diffused irradiance. By just tilting the trays by 20°, it allows more light to reach inwards, which would allow for a longer width of the trays. This in turn also increases the total yield of a tray in a limited space. This discovery can be utilised for the layout and arrangement for indoor VFs that relies on external solar irradiance for a light source and is proof that crop yield can be increased without adding extra energy consumption.

Highlights

- Food engineering education in solar based VF system.
- Discuss challenges faced in modern land-scarce urban agriculture farms.
- TracePro 2019 for raytracing used in conjunction with ASHRAE sunlight modelling method.
- Tray width to height ratio is highly dependent on, to maximise crop yield while retaining amount of sunlight received for indoor vertical farming.
- Tilting of crop trays towards the sunlight is the most cost effective and energy saving way to maximise solar flux and space usage.
- External direct solar irradiance flux on crop trays decreases as tray inclination angles increases, but overall solar irradiance increases as steeper angles results in increased reflected solar irradiance.
- Capturing of reflected and diffused irradiance should be prioritised rather than maximising direct irradiance.

1.Introduction

Traditionally, the conventional farming method was a favoured approach to produce crops in large quantities. It is a farming system that requires the use of synthetic chemical fertilisers, pesticides, herbicides, heavy irrigation, and other continual inputs for crops production. This method, which is also commonly known as industrial agriculture, boasts a high crop yield at the expense of being energy and resource extensive. In Singapore, due to its limited land space, a more innovative and energy efficient farming system will be needed to enable us to grow as much of our own supply of crops.

Urban Vertical Farming (VF) is no new idea and has been actively researched on in many countries. It provides us with a solution when it comes to growing our own crops in a limited area as theoretically, a VF is only limited by how high the building can be built. Apart from solving the land issue, there are many more advantages that comes with it. As a start, water losses would be kept to a minimum as the usage of water is controlled and transpired water can be reused, further saving the usage. No pesticides would be required as in a controlled environment, there would be no pests to damage the crops, allowing the crops to grow more organically. The usage of artificial lighting also mean that crops can grow throughout the year and will not be hindered by weather, allowing maximum crop yield. Dr. Despommier of Columbia University believes that the ways indoor farming should be done is through tall multi-storey structures. He claims that one VF with an architectural footprint of one square city block and rising to 30 stories could provide enough nutrition to comfortably accommodate the needs of 10,000 people with the currently available technologies (Despommier and Ellingsen, 2008). In addition, it is stated in a research that VF will lower overall carbon dioxide output by 67 – 92% as compared with greenhouse gases, which is in line with Singapore's Clean and Green campaign (Graamansa *et al*, 2018).

However, one of the more prominent issues highlighted for VF is the amount of energy required to maintain the environment within the levels. A lot of energy would be required to produce artificial lighting for the plants as sunlight would not be ample due to design constraints (Kalantari *et al,* 2017). Energy would also be required by air pumps to maintain the temperature and humidity of the room, to ensure that the plant can grow in the optimum condition. A possible way to reduce the energy consumption would be to find a way in which solar irradiance can still be utilized, by optimizing the reflectivity of the surfaces within the farm, adopting an 'open' concept by using glass walls instead of opaque structural walls, or simply by the arrangement of the plant trays. Heat from the irradiation of the Sun could be harnessed to heat up floors that require the extra energy to maintain the set temperature. However, the presence of an external heat source combined with waste heat from the operating systems may prove to be counterproductive instead.

The aim of this paper would be to identify how different tray placement designs would affect the solar irradiance received. The variable design parameters are the position, width, angle of tilt of the trays with respect to the windows and reflectivity of the surfaces within the room. These designs are supported by a modelling of the Sun's position with respect to the rooms at various times of the day to observe the pattern of irradiance, such that a maximum flux can be captured in a day. A ray tracing software will be utilized to generate accurate data for the charts. An estimation of the ratio of heat energy contributed by the Sun to the waste heat energy generated by the operating systems of a VF would also be generated, as means to determine if urban VF should adopt an 'open' concept.

1.1.Motivation

Singapore is a small city with very limited land available for agriculture and 90% of the food we consume are imported from other countries. There is a growing demand in fresher food and food grown locally could be one of the methods to solve this. Locally produced food would also be cheaper and more affordable due

to the reduction in costs from the transportation process. We would also need to cultivate our own crops as a backup to increase the food security of Singapore particularly for the Covid-19 lockdown periods.

As Singapore progresses to be a smart and self-sustaining nation, having an energy efficient farming system here may serve as a potential case study for other countries to learn from, due to the compact nature of our landscape. This can boost our country's presence worldwide in the utilisation of VF systems. So far, there has also been a lack of research that evaluates the energy consumed by VF locally and the best possible way for plant arrangement. To ensure that urban VF becomes a suitable and sustainable way of farming in Singapore, it is crucial to gain insights from various related research papers and experienced organisations. Therefore, this article may serve to provide some groundwork for this area of study.

2. Materials and methods

Before we can state the assumptions for the simulation parameters, we would first need to find out the total energy coming from the Sun throughout the year. As we know, the Sun radiates a tremendous amount of energy throughout the year and it contributes to most of the heat generation in a system. The position and angle of the sun with respect to the simulation plays an important role as it determines the angle at which the rays strike the surface, which in turn affects the amount of energy received and transferred into the room.

These approaches will be used in conjunction to derive the total energy received by the VF and to subsequently determine the suitability of the design.

2.1.Mathematical Evaluation (with ASHRAE)

A comprehensive set of calculations would be required to evaluate the results required for the actual simulation of the VF design. The approach is split into 6 steps:

Determination of Solar Declination Angle (SDA) in Singapore as test example

Determination of Solar Incidence Angle (SIA) to the normal of the walls

Evaluation of Total Solar Irradiation per square unit on the walls

Evaluation of Total Heat Transfer through the walls into the room

Addition of both latent and sensible heat within a room to determine cooling load

Energy cost analysis

For the determination of the SDA and SIA, the ASHRAE Model would be used to obtain their values, respectively. This alternative eliminates the need for an actual equipment, which is highly cost. This model's algorithm provides a simple method to predict the position of the Sun relative to Singapore and even allows for rough estimations of the solar irradiation (Abouhashish, 2017; Yadav and, Chandel, 2014). Also, ASHRAE Model allows for the estimation of values at any time of the year. However, if this study is to be continued for further refinement and research, it would be necessary to do more literature review for the determination of the best model (e.g. Perez), or getting an equipment to generate a data log for Solar Irradiation on Singapore.

The purpose of doing a manual calculation of heating load is to obtain the irradiance energy which is required by Trace Pro 2019 software. Those energy readings can then be reflected on the simulation parameters in TracePro to obtain respective flux on the tray readings, which in turn can be converted to photometric units. The values are obtained for any single time or date in the year and the location can be customizable as mentioned above.

2.2.Trace Pro 2019

This software is a commercial optical engineering software program used for designing and analysing optical and illumination systems. The program's graphical user interface (GUI) is 3D CAD-based creating a virtual prototyping environment to perform software simulation before manufacture. Users can import models created with SolidWorks into TracePro to simulate lit appearance of illumination or lighting systems and trace bitmap images through optical systems to check for uniformity. Thermal energy effects can also be captured by this code.

TracePro is used here to determine energy distributions on the tray surfaces and tracking of the volume flux in the control volume. Through TracePro, a light source will be created and will mimic the properties of Sunlight entering a room. It also plays a key role in the optimisation of the room design, ranging from the distance of trays from the windows, to the height in between each tray including the angle of orientation for the trays.

The results generated by TracePro come in the form of both visual data and numerical data and they can be exported out with ease.

3.Results and discussions

3.1.Determination of Solar Angle of Declination (δ)

The Solar Angle of Declination is an angle that is dependent on the date of the year Earth is at. The declination of the Sun is the angle between the equator and a line drawn from the centre of the Earth to the centre of the Sun. It follows the relation as shown below:

$$\delta = 23.45° \times \sin\left[\frac{360(284+n)}{365}°\right] \quad (1)$$

3.1.1.Determination of Solar Altitude Angle (β) & Solar Azimuth (∅)

The Solar Altitude Angle (β) is an angle that is between the Sun's rays and the horizontal of a point. It is used to model the vertical angle of the Sun's ray entering the control volume. It depends on three main parameters, the Hour Angle (h), Latitude (l) of point and Solar Angle of Declination (δ). The hour angle is also dependant on the solar time t_s, e.g. (1pm = 13 and 2pm = 14) as below:

$$h = 15(t_s - 12)\ hrs \quad (2)$$

$$sin\beta = coslcoshcos\delta + sinlsin\delta \quad (3)$$

The Solar Azimuth (∅) is required to determine the angle between the projection of the Sun's ray on the horizontal plane and the south direction. It will be useful in modelling the horizontal angle of the Sun's ray entering the control volume.

$$cos\emptyset = \frac{sin\beta sinl - sin\delta}{cos\beta cosl} \quad (4)$$

3.1.2.Evaluation of Total Solar Irradiation per square units on the walls

The Total Solar Irradiation entering the control volume is simply the addition of the Normal Direct Irradiation (G_D) , Diffuse Radiation ($G_{d\theta}$) and Reflected Radiation (G_R). Their formulas are given as:

$$G_D = C_N * Ae^{-\left(\frac{B}{sin\beta}\right)} * cos\theta \quad (5)$$

$$G_{d\theta} = \frac{C * Ae^{-\left(\frac{B}{sin\beta}\right)} * F_S}{{C_N}^2} \quad (6)$$

$$G_R = \left[C_N * Ae^{-\left(\frac{B}{sin\beta}\right)} * sin\beta + \frac{C * Ae^{-\left(\frac{B}{sin\beta}\right)} * F_S}{{C_N}^2}\right] * \rho * F_S \quad (7)$$

where

C_N is Coefficient of Clearness of Sky (Assumed as 1)

A is the Apparent Solar Radiation taken from the ASHRAE Table 1.

B and C are correction factors from ASHRAE Table

θ is $\cos^{-1} sin\beta$

F_S is the view factor (0.5 for the current control volume)

ρ is the reflectance of the object (0.2 for the current control volume)

Table 1. ASHRAE solar data (2001)

EOT, Declination, Extraterrestrial Solar Data Tables (ASHRAE 2001)

Month	G_{SC} W/m²	EOT min	δ °	A W/m²	B	C
Jan	1416	-11.2	-20.0	1230	0.142	0.058
Feb	1401	-13.9	-10.8	1215	0.144	0.060
Mar	1381	-7.5	0.0	1186	0.156	0.071
Apr	1356	1.1	11.6	1136	0.180	0.097
May	1336	3.3	20.0	1104	0.196	0.121
Jun	1336	-1.4	23.45	1088	0.205	0.134
Jul	1336	-6.2	20.6	1085	0.207	0.136
Aug	1338	-2.4	12.3	1107	0.201	0.122
Sep	1359	7.5	0.0	1151	0.177	0.092
Oct	1380	15.4	-10.5	1192	0.160	0.073
Nov	1405	13.8	-19.8	1221	0.149	0.063
Dec	1417	1.6	-23.45	1233	0.142	0.057

3.2.Results & Findings from Trace Pro 2019

3.2.1.Model Design A with 1:2 Height to Width Ratio (@0800hr)

Fig. 1 and Table 2 presents the basic schematic view and design variables & parameters for our models used.

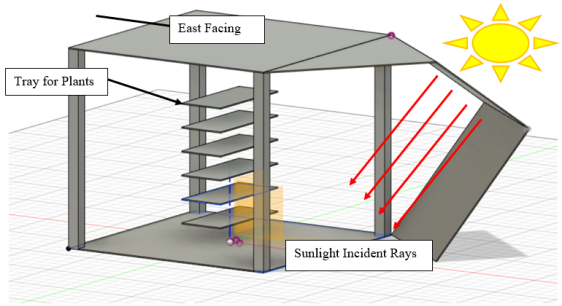

Figure 1. Model A 1:2 Height to Width Ratio @ 0800hrs

Table 2. Design A Variables & Parameters used

Tray for Plants	
Width	80mm
Length	180mm
Thickness	2mm
Distance in between	40mm
Distance from Window	160mm
Base of Floor	
Length	400mm
Width	400mm
Height	350mm
Sun Ray	
Emission Type	Irradiance (Visible Light Spectrum)
Power	737.84 W/m^2
Solar Altitude Angle	27.93 Degrees
Solar Azimuth Angle	25.65 Degrees

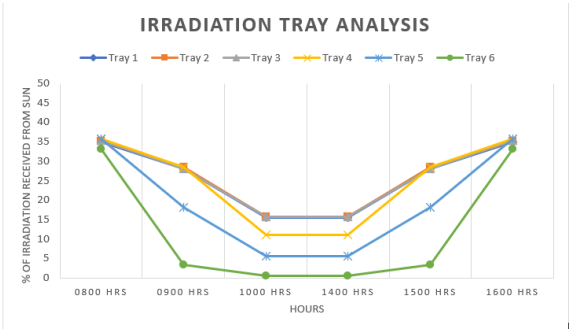

Figure 2. Irradiation Tray Analysis (Design A)

3.2.2.Shaded Area for Design A

To maximise space usage, the space between two trays is kept at 40 units as lettuce only grow to an average height of 12 inches. Coupled with the sunlight declination angle, part of the tray will be shaded from direct irradiance. The shaded area can be related to the distance between each tray by *Eq. (8)* as shown in Fig. 3.

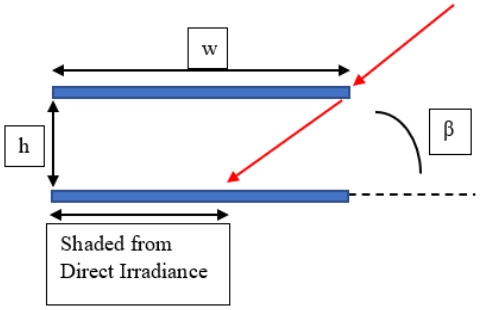

Figure 3. Schematic Side view of Trays layout

$$\left(1-\left(\frac{h}{w}\right)*\left(\frac{1}{tan\beta}\right)\right)*100\% = \%\ area\ shaded \qquad (8)$$

As the solar declination angle increases throughout the day, the percentage of shaded area on each tray are increased. This reflects the steady decrease of irradiance flux on each of the tray surfaces as seen from Fig. 2. From Eq. 8, it can be intuitively inferred that the more the percentage of shaded area, the lesser the irradiance flux on the tray surface (Table 3).

Table 3. Average Flux Decrease (Design A)

	0800 hrs	0900 hrs	1000 hrs
Solar Declination Angle	27.93	41.21	53.61
Average % Flux	35	22	11
Average Flux Decrease	Approx. 12% per Hour		

Table 4. Photosynthetic Proton Flux Density (PPFD) (Design A)

S/N	Average Flux Received (W)	Average Flux Received per metre ($\frac{W}{m^2}$)	PPFD ($\frac{\mu mol}{sm^2}$)
Tray 1	2.75	191	447
Tray 2	2.78	193	451.6
Tray 3	2.77	192.4	450.2
Tray 4	2.65	184.3	431.3
Tray 5	2.1	146.1	341.9
Tray 6	1.32	91.4	213.9

From Table 4, the PPFD values obtained were suitable for the growing of lettuce, which means indoor growing of plants near to the

window would be possible with natural light alone.

It can also be inferred from Fig. 2 that the irradiance flux on the lower trays changes less drastically as compared to trays closer to the top. The drop in flux on each tray at different time is also more stable on the lower trays as compared to those at the top. It would be suggested that the trays be placed as close as possible to the windows.

At higher solar declination angles, trays 5 and 6 showed a drastic change in irradiance as compared to the other trays. This suggests that trays closer to the top are more sensitive to changes in the angle of declination and if likely, should be replaced by trays perpendicular to the floor (see Fig. 4).

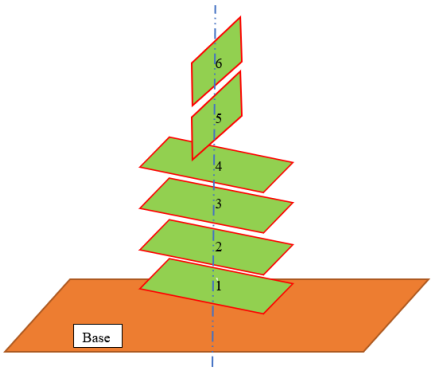

Figure 4. Rough illustration of Trays Layout

3.2.3.Model Design B with 1:3 Height to Width Ratio (@0800hr)

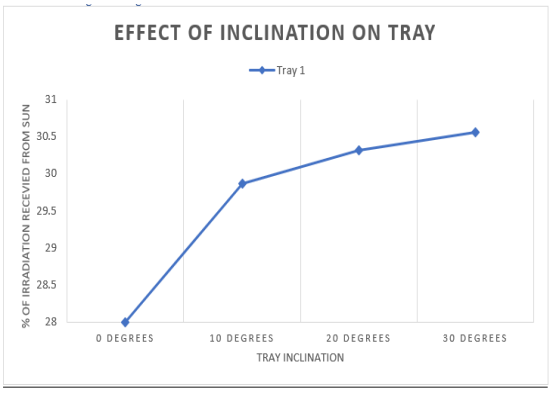

Figure 5. Relationship between Irradiance Flux Received to Angle of Inclination on Tray 1 (Design B)

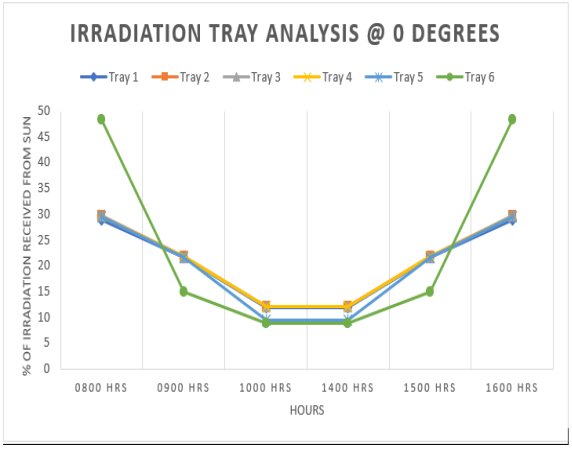

Figure 6. Irradiation Tray Analysis @ 0˚ (Design B)

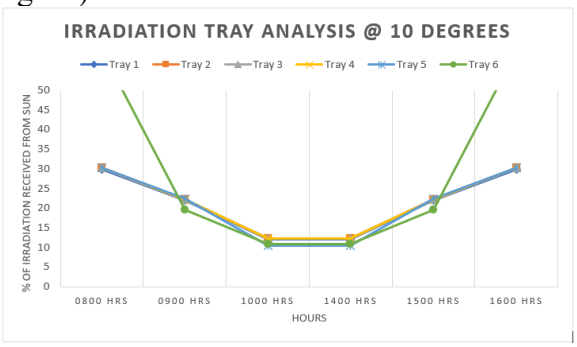

Figure 7. Irradiation Tray Analysis @ 10˚ (Design B)

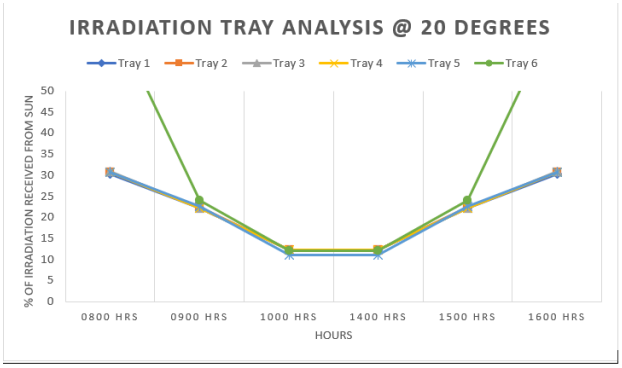

Figure 8. Irradiation Tray Analysis @ 20˚ (Design B)

Since the average lettuce requires a Photosynthetic Proton Flux Density (PPFD) of 196.8 $\frac{\mu mol}{sm^2}$, the results in Table 5 reveal that there is an adequate amount of natural sunlight to sustain its growth.

Table 5. Photosynthetic Proton Flux Density (PPFD) of Trays @ 20˚ Tilt (Design B)

S/N	Average Flux Received (W)	Average Flux Received per metre ($\frac{W}{m^2}$)	PPFD ($\frac{\mu mol}{sm^2}$)
Tray 1	3.4	157.4	368.3
Tray 2	3.45	159.7	373.7
Tray 3	3.44	157.4	368.3
Tray 4	3.44	157.4	368.3
Tray 5	3.35	155.1	363
Tray 6	4.8	222.2	520

Comparing the values between Tables 4 and 5, Tray 6 from Design B received an equivalent of 520 PPFD whereas Design A only received significantly lesser at 213.9 PPFD. A small portion of the increase can be attributed to the increase in tray width on Design B, but a large portion of it was caused by the tilting of the trays. The tilting combined with no shading, simply allowed Tray 6 at the top to be exposed to a higher irradiance flux from the sun.

3.3.Shaded Area for Design B

For design B, due to the addition of inclination and width of trays (Fig. 9), it is expected that the shaded area of the trays beneath will be affected more. This means that intuitively there would be smaller amount of irradiance flux on the surface of the trays, resulting in smaller values.

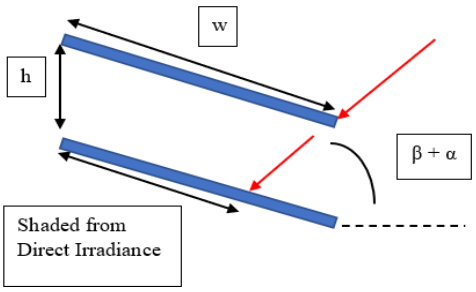

Figure 9. Schematic Side view of Trays layout with an extra inclination angle α

$$\left(1-\left(\frac{h}{w}\right)*\left(\frac{1}{\tan(\beta+\alpha)}\right)\right)*100\% = \%\ area\ shaded \qquad (9)$$

Table 6. Relationship between Angle of Inclination and Shaded Area of Trays (Design B)

Angle of Inclination (˚)	Hour (hrs)	Angle (˚)	Shaded Area
0	0800	27.93	37.12%
	0900	41.21	61.94%
	1000	53.61	75.43%
10	0800	27.93	57.23%
	0900	41.21	73.21%
	1000	53.61	83.46%
20	0800	27.93	69.91%
	0900	41.21	81.68%
	1000	53.61	90.20%

This is supported by the data obtained in Table 6, with the whole tray almost in the shade at 20˚ inclination 1000 hrs. However, from Table 7 and Figs. 5 to 8, it can be interpreted that increasing the angle of inclination of the trays increases the irradiance flux on the trays. It can be argued that with the increase of angle, the exposed surface has a larger angle normal to the irradiance which results in the higher calculated flux. On closer inspection what can be noticed is that the 'red' region (Figs. 10 & 11) of the trays reduces in area – which is supported by the larger shaded area.

On the other hand, there is an increase in the reflected and diffuse irradiance that is casted normal to the shaded area of the trays. This phenomenon is also seen on the trays at other timings of the day, which further reinforces the theory that a higher tilt angle can be utilised effectively to bounce off irradiance to the unshaded areas of the tray. This suggests that design B can be likely adopted for above ground 'open' VF to optimize the growing of crops.

The increase in flux on each tray is however not as significant as expected, which can be attributed to the simulation parameter being used. The reflectivity of the tray surface was set to 0.1 so as to set the contrast between the area under the direct irradiance and the parts affected by reflected irradiance. Therefore, it is more important to focus on the relative changes than the actual value itself as the values act more as an extremely conservative result. It is notable that as the angle of inclination increases, the bottom 4 trays tend to receive almost the same amount of flux regardless of their position relative to the ground. Tray 6 is more heavily affected by changes in the inclination.

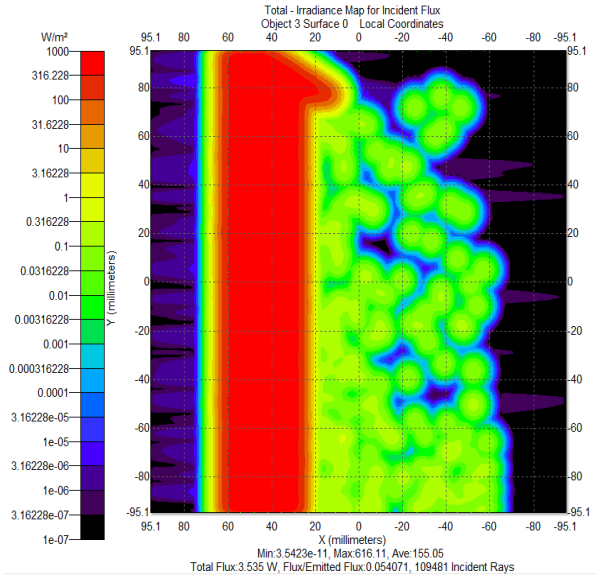

Figure 10. Total irradiance map for incident flux of Tray 3 @ 0900hrs 10˚ Incline

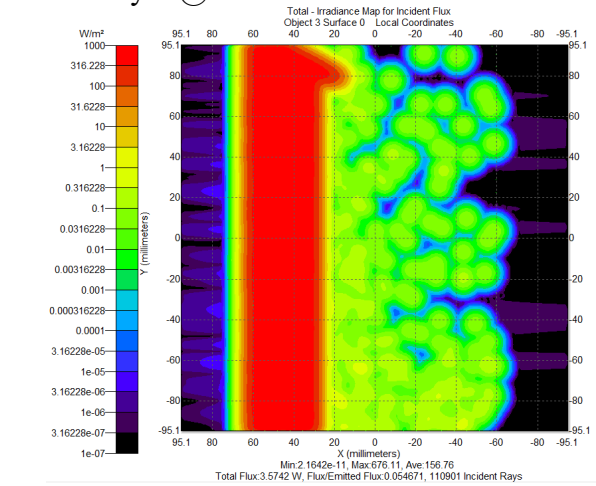

Figure 11. Total irradiance map for incident flux of Tray 3 @ 0900hrs 20˚ Incline

Table 7. 0˚ Inclination, 10˚ Inclination and 20˚ Inclination Comparison

0˚	0800 hrs	0900 hrs	1000 hrs
Solar Declination Angle	27.93	41.21	53.61
Average % Flux	32.5	20.6	11.1
Average Flux Decrease	Approx. 10.7% per Hour		
10˚	0800 hrs	0900 hrs	1000 hrs
Solar Declination Angle	27.93	41.21	53.61
Average % Flux	35.2	21.8	11.7
Average Flux Decrease	Approx. 11.75% per Hour		
20˚	0800 hrs	0900 hrs	1000 hrs
Solar Declination Angle	27.93	41.21	53.61
Average % Flux	37.0	22.6	12.1
Average Flux Decrease	Approx. 12.45% per Hour		

4. Conclusions

By comparing the findings between designs, A and B, it can be determined that both designs allow for normal growth of crops (lettuce) in an urban setting with just natural lighting alone. However, the main difference between designs A and B is that the latter allows for a much higher surface area for whilst taking the same amount of vertical space. Design B allows for a higher yield due to the increase in surface area from the increase width of tray and at the same time, compensates the loss in irradiance due to shading by orientating the trays at an angle. By doing this seemingly small tweak, it optimises the amount of irradiance received by the trays, by taking advantage of reflected and diffused irradiance on the tray surfaces. This slight tweak permits the sunlight to reach further into the trays, ensuring that they receive adequate sunlight. This is done so with extremely restricted test parameters and it is certain that more optimistic results would be shown if plausible parameters are utilised for actual tests. Thereafter, by optimizing the solar irradiance through the assistance of the ray-tracing software, it can be illustrated that growing crops on trays in a confined urban setting is possible with following considerations:

Trays to be placed as close as possible to window due to fenestration.

Trays to be oriented at maximum 20 degrees if possible.

Ideally, top 2 trays could be perpendicular to the floor (at the sacrifice of crop space).

Underside of trays to be coated with high reflectivity material to maximise diffused and reflected irradiance.

Tray height to width ratio should be maintained near 1/3.

Trays should not be rotated but rather supported by strategically placed LED lights.

Finally, this paper could serve as learning purposes for basic educational entry point readers to the design configurating of solar based vertical farming. We used direct light point source in this TracePro software for simulations of flux intensities and may not directly applicable to more advanced VF designs such as the Agri-PV concept with the Daily Light Integral (DLI) / Photosynthetic Photon Flux Density (PPFD) (Zhang and Ng, 2021, in-press) over a full annual solar cycle and including the helio/photo-tropism in crops. The limitation of this work is that we have not conducted experimental validation.

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SOME QUALITATIVE PROPERTIES OF COMMON CARP (*CYPRINUS CARPIO*, L. 1758) FROM DIFFERENT AQUATIC ENVIRONMENT IN N. MACEDONIA

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ABSTRACT

The main purpose of this study was to consider some qualitative properties of farmed and wild common carp (*Cyprinus carpio*, L. 1758) from waters in N. Macedonia. The qualitative properties of the common carp were established by determination of the chemical composition of the fish meat, the energy value of meat, and the microbiological analysis for the total number of microorganisms on fish skin and presence of *Salmonella* sp. and *Listeria monocytogenes*. The results obtained during the examination of the chemical composition of the common carp meat from the aquaculture shown the mean value of 71.27 % water, 19.98 % proteins, 4.23 % fats and 0.98 % ash. The results of the common carp meat from open waters were as follows: 76.03 % water, 10.02 % proteins, 2.92 % fats, and 1.06 % ash. The differences in the values of fat content between fish meat from aquaculture and open waters carp are statistically significant on level ($p<0.05$). The differences in the values of protein content are statistically significant on level ($p>0.05$). The significantly higher energy value is established in the common carp meat from aquaculture (507.817 kJ/100 gr) compared to the meat from open waters (285.706 kJ/100 gr), as a result of significantly higher values of fat and protein content in aquaculture common carp.

Such results for examined qualitative parameters in cultivated and autochthonous common carp from N. Macedonia are the first published results for our country.

1. Introduction

Cyprinid fish species, including common carp (*Cyprinus carpio* L. 1758) are predominant fish species in the world's aquaculture, accounting for 54% of total fish production (FAO, 2006).

Common carp (*Cyprinus carpio* L. 1758) is one of the most valuable and consumed fish species in N. Macedonia. It is the predominant fish species in the cyprinid aquaculture facilities in our country and successfully bred in aquaculture (warm water - cyprinid fish farms and cage farms) where it covers 60 - 80% of the total fish population. The dominant form of aquaculture production of common carp is the semi-intensive farming system, where the fish diet is based on a combination of natural foods and complementary foods (cereals - wheat, corn, and barley).

Regarding the open waters, common carp is one of the most important components in the overall ichthyomass of Prespa Lake.

From a nutritional point of view, fresh fish and fish products are important for proper nutrition and prevention of health in humans. The high nutritional value of fish meat is due to

the favorable composition and ratio of proteins, fats, minerals, and vitamins, as well as the significant presence of unsaturated fatty acids, especially n-3 PUFA (Connor, 2000; Sidhu, 2003). The composition of protein in fish is better compared to the protein composition of other animals, which is mainly due to the more favorable amino acid composition and the number of free amino acids (Toppe et al., 2007; Buchtová et al., 2010). Fish proteins contain all the essential amino acids for the human body and can be used as the only source of protein in the diet (Vladau et al., 2008). In terms of fat, mammal meat contains a higher percentage of fat compared to fish meat (Saičić et al., 2010), but in terms of composition, fish fat differs from fat in mammals due to its higher content of monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids. The fat content of fish varies depending on the fish species, the season, as well as the type of food (Guler et al., 2008; Čirković et al., 2011).

Ljuboević et al. (2013) in their research on the chemical composition of carp (average weight 1420 g) of open water (Danube River) in Serbia received the following results: 73.73% water, 16.69% protein, 7.13% fat, and 0.88% ash.

Yeganeh et al. (2012) conducted a study on the chemical composition of aquaculture carp and open water carp during all four seasons and found that the fat and protein content in carp samples decreased from summer to spring, so in aquaculture carp, 17.6% protein in summer and 15.9% protein in spring were determined, while in open water carp 18.2% protein in summer and 17.9% protein in spring were determined. In terms of fat, in the samples of carp from aquaculture 5.1% fat in summer and 1.5% fat in spring were found, while in open water carp, 3.8% fat in summer and 2.8% fat in spring were found.

Čirković et al. (2012) in their research on the chemical composition of carp grown in the Echka fishpond, Serbia, determined the presence of 16.21% protein, 2.42% fat, 80.36% water and 1.02% ash.

Afkhami et al. (2011) in their research on the chemical composition of carp from a fish farm in Iran obtained the following results: 15.2% protein, 3.53% fat, 1.5% ash and 75.48% water.

According to Čirković et al. (2011), the chemical composition of a three-year-old carp raised in semi-intensive conditions and fed with barley, corn and wheat with 40:30:30 was: 70.67% water, 15.81% protein, 11.73% fat and 0.93% ash. The percentage of water, protein, fat and ash in a three-year-old carp fed with a complete pelleted food was: 70.94, 17.68, 10.41 and 0.94, respectively.

Marcu et al. (2010) in their research on the chemical composition of carp weighing 785 - 2010 g, from aquaculture in Romania, obtained the following results: 76.06% water, 17.79% protein, 5.07% fat and 1.07% ash.

Bud et al. (2008) in their research on the chemical composition of open water carp from Romania, obtained the following results: 73.22% water, 16.6% protein, 8.97% fat and 1.20% ash.

Hadjinikolova (2008) in her research on the chemical composition of carp from waters in Bulgaria received the following results: 74.55% water, 16.21% protein, 8.30% fat and 0.94% ash, while the energy value of fish meat was 717.3 kJ.100g-1.

The main purpose of this study was to consider some qualitative properties of farmed and wild common carp (*Cyprinus carpio*, L. 1758) from waters in N. Macedonia. This means that the common carp is grown at different ambient conditions in the aquatic environment, as well as different feed.

2. Materials and methods

2.1. Materials

Examinations in this study were performed on samples of common carp (*Cyprinus carpio*, L. 1758) from two different aquatic environments: aquaculture facility (cyprinid fish farm) and open waters (natural lake). Carp samples were taken in the spring season. The average mass of common carp samples from aquaculture was 1200 g, while the average

length, 42 cm. The average mass of common carp samples from the lake was 1450 g, while the average length, 70 cm.

The cyprinid fish farm is located near the city of Bitola (N. Macedonia), i.e. the southeastern part of Pelagonia. It was built in 1960/61 by enclosing part of the old riverbed of Crna Reka with two embankments. It covers an area of 170 ha and it is the biggest cyprinid fish farm in N. Macedonia. The land on the old riverbed of Crna Reka is very fertile, which contributes to the fish farm is very productive, i.e. rich in zoo and phytoplankton. The traditional approach to carp breeding is based on food that is naturally present in the fish farm (zooplankton and benthos, i.e the flora and fauna at the bottom), but the diet is supplemented with unprocessed cereals (wheat, corn, barley).

Figure 1. Cyprinid fish farm "Zhabeni", Bitola (N. Macedonia) (original)

Prespa Lake is the second - largest natural lake in N. Macedonia. In terms of the composition of the fish settlement, it is typically a cyprinid lake. The autochthonous ichthyofauna in Prespa Lake consists of the following fish species: common carp, Pelister trout, eel, Prespa gomnushka, belvica, black barbell, Prespa scab, Prespa grunec, Prespa maple and Prespa pinch.

Figure 2. Prespa Lake (N. Macedonia) (original)

2.2. Methods

During examinations, the following methods for determination of the chemical composition of fish meat were used:

- Determination of moisture content - ISO 712:2009;
- Spectrophotometric determination of total nitrogen according to Kjeldahl - HACH DR 400 procedure method 2410;
- Determination of the total fat by gravimetric method (Soxhlet extraction) - AOAC method 2003.6;
- Determination of ash in an oven at 700°C - ISO 3593:1981;

Methods for microbiological analyses of fish meat that were used:

- Horizontal method for detection and enumeration of *Listeria monocytogenes* - ISO 11290 - 1:2008;
- Horizontal method for detection and enumeration of *Salmonella* sp. - ISO 6579 - 2008;
- Horizontal method for the enumeration of microorganisms - ISO 4833:2003.

Methods for physical - chemical properties of the water that were used are:

- pH determination - ISO 1052:1994;
- Chloride determination - ISO 9297:1989;
- Spectrophotometric determination of nitrates - HACH DR 400 procedure Method 8039;
- Spectrophotometric determination of iron - HACH DR 400 procedure Method 8365;
- Spectrophotometric determination of nitrites - HACH DR 400 procedure Method 8507;
- Turbidity determination of translucency - ISO 7027:1999;
- Spectrophotometric determination of ammonia - HACH DR 400 procedure Method 8038;
- Determination of chemical oxygen demand, Merck Method Spectroquant 1.18752.0001;
- Total nitrogen determination - Merck Method Spectroquant 1.14537.0001.

Methods for microbiological analyses of water that were used:

- Detection and enumeration of coliform bacteria and *Escherichia coli* - ISO 9308 - 1:2000;

- Detection and enumeration of intestinal enterococci and *Streptococcus faecalis* - ISO 7899 - 2:2000.

The energy value (EV) of common carp meat was calculated according to the formula:

EV (кJ/100g) = proteins (%) x 17.16 + fats (%) x 38.96 (Vitcenko et al., 1981).

Standard descriptive statistical analyses (Microsoft Office Excel 2010, Data Analysis ToolPak and t - test) were used for results processing.

3.Results and discussions

The qualitative properties of the common carp were established by determination of the chemical composition of the fish meat, the energy value of meat and the microbiological analysis for the total number of microorganisms on fish skin and presence of *Salmonella* sp. and *Listeria monocytogenes*. The main purpose of the research produced additional analyses that determine the physical - chemical properties and also a microbiological analysis of the water in which the common carp resides.

Considering the results of the physical - chemical and microbiological analysis of the water samples from the fish farm and Prespa Lake, the following results were obtained:

Table 1 Physical – chemical properties of water samples

Parameters	Fish farm	Prespa Lake
Temperature	14.2°C	24°C
Dissolved oxygen	10.7 mg/l	10.1 mg/l
Represent of oxygen - saturation	103 %	105 %
5-day biochemical consumption of O_2 at 20 °C	1.00 mg/l	2.60 mg/l
Chemical oxygen consumption	5.00 mg/l	5.00 mg/l
Suspended matters	2 mg/l	1.86 mg/l
Dry residue of filtered water	225.0 mg/l	211.0 mg/l
pH	8.68	8.85
Nitrites	0,010 mg/l	0,000 mg/l
Nitrates	1.97 mg/l	0.000 mg/l
Chlorides	21.30 mg/l	15.60 mg/l
Ammonia	0.020 mg/l	0.020 mg/l
Electro conductivity	402.000 ms/cm	398.000 ms/cm
Total phosphorous	0.005 mg/I	0.004 mg/I
Total nitrogen	0.236 mg/I	0.193 mg/I

Table 2 Microbiological properties of the water samples

Parameters	Fish farm	Prespa Lake
The probable number of thermo-tolerant coliform bacteria in 100 ml	10	10
Streptococcus of fecal origin in 100 ml	50	0

The water quality is a combination of chemical, physical and biological parameters. It`s properties affect the general fish condition and determines the fish growth and health. As a result, water quality is a major factor to be taken into account when planning fish breeding.

Based on the physical - chemical and microbiological properties of the water samples from a fish farm and Prespa Lake, the water is classified into class II (according to the Regulation on water classification Official Journal of RM 18/99), which is allowed for fish production.

Considering the results of the microbiological analysis for the total number of microorganisms on fish skin and the presence of *Salmonella* sp. and *Listeria monocytogenes*, the following results were obtained:

Table 3 Microbiological analysis of common carp

Parameters ($\bar{x}$)	**Total number of microorganisms (log CFU/cm^2)**	***Salmonella* sp.**	***Listeria monocytogenes***
Fish farm	4.26	0	0
Prespa Lake	3.66	0	0

Legend: $\bar{x}$ - mean value

In terms of determining the total number of microorganisms on the skin of common carp, in our study average value of 4.26 (a fish farm) and 3.66 (Prespa Lake) log SFU/cm2 were considered, which is in correlation with the findings of Adams and Moss (2008) which concluded that the total number of microorganisms on the fish skin surface ranged from 2.00 - 7.00 log CFU/cm2.

Considering the results of the chemical composition and energy value of the common carp meat from aquaculture and open waters, the following results were obtained:

Table 4 Chemical composition (%) of the common carp meat from aquaculture and open waters

Chemical components	**Fish farm**				**Prespa Lake**			
	$\bar{x}$ ± SD	**min**	**max**	**CV**	**$\bar{x}$ ± SD**	**min**	**max**	**CV**
Water	71.266 ± 0.499	70.60	71.80	0.007	76.033 ± 1.400	74.20	77.60	0.018
Proteins	19.983 ± 0.103	19.85	20.10	0.005	10.020 ± 0.059	9.96	10.10	0.005
Fats	4.233 ± 0.213	3.94	4.44	0.050	2.920 ± 0.169	2.75	3.15	0.057
Ash	0.983 ± 0.071	0.91	1.08	0.072	1.064 ± 0.005	1.06	1.07	0.004

Legend: $\bar{x}$ - mean value; SD – standard deviation; min – minimum value; max – maximum value; CV – coefficient of variation.

Table 5. Comparative indicators of the chemical composition (%) and energy value (κJ/100 g) of the common carp meat from aquaculture and open waters

Parameters	Water	Proteins	Fats	Ash	Energy value
Fish farm	**71.266** ± 0.499	**19.983**[a] ± 0.103	**4.233**[b] ± 0.213	**0.983** ± 0.071	507.l817
Prespa Lake	**76.033** ± 1.400	**10.020**[a] ± 0.059	**2.920**[b] ± 0.169	**1.064** ± 0.005	285.706

[a]The differences in the values with the same superscripts are statistically significant on level p>0.05
[b]The differences in the values with the same superscripts are statistically significant on level p<0.05

In fish farms, common carp use natural and additional foods. The common carp feed additionally only during the summer. In our study, the diet of farmed carp is based on food that is naturally present in the fish farm (zooplankton and benthos, i.e. the flora and fauna found at the bottom), but it is supplemented with unprocessed cereals (wheat, corn, barley).

Regarding the open waters, the benthic zone is an essential part of the lake's biotope. Nutrients, organic matter and microorganisms are present here at a much higher density compared to those in fish farm water. The common carp, in any case, covers its food needs from the benthos of the fish farm or lake, so their characteristics affect the meat quality, directly through their consumption and indirectly through the water.

The results obtained during the examination of the chemical composition of common carp meat from the aquaculture shown the mean value of 71.27 % water, 19.98 % proteins, 4.23 % fats and 0.98 % ash. On the other hand, the results of the common carp meat from open waters were as following: 76.03 % water, 10.02 % proteins, 2.92 % fats and 1.06 % ash.

The results obtained from our study of the chemical composition of aquaculture carp are in correlation with the findings of Yeganeh et al. (2012) (17.6% - 15.9% protein, 5.1% - 1.5% fat, 76.7% - 81.4% water, 0.6% - 1.2% ash); Čirković et al. (2012) (16.21% protein, 2.42% fat, 1.02% ash and 80.36% water); Afkhami et al. (2011) (15.2% protein, 3.53% fat, 1.5% ash and 75.48% water); Čirković et al. (2011) (70.67% water, 15.81% protein, 11.73% fat and 0.93% ash) and Marcu et al. (2010) (water 76.06%, protein 17.79%, fat 5.07%, ash 1.07%).

The results obtained from the study of the chemical composition of open water carp are in correlation with the results of Ljuboević et al. (2013) (73.73% water, 16.69% protein, 7.13% fat and 0.88% ash); Bud et al. (2008) (73.22% water, 16.6% protein, 8.97% fat and 1.20% ash) and Hadjinikolova (2008) (74.55% water, 16.21% protein, 8.3% fat and 0.94% ash).

In our study, the common carp meat from aquaculture contained significantly less water (71.27%) compared with the one from open waters (76.03%). Fat content in the common carp from aquaculture was 4.23 %, which is significantly higher compared with the one from open waters (2.95 %). In our tests, the differences in the values of fat content are statistically significant on level (p<0.05).

Kaushik (1995) considered that the fat content in fish meat is directly related to nature and fat content in the food, while the water content of fish meat is inversely proportional to the fat content. The inversely proportional relationship between fat and water content in farmed and wild common carp has also been established by Love (1970), which is in correlation with our results.

Also, significantly higher values for the protein content in the common carp from aquaculture (19.98%) compared to the common carp from open water (10.02%) were observed. In our tests, the differences in the values of the protein content of common carp from aquaculture and open waters are statistically significant on level (p>0.05). The average

amount of ash is slightly higher in common carp from open waters (1.06 %) compared with the one in aquaculture (0.98 %).

The data from the literature reviews related to the chemical composition of common carp differ, especially regarding the fat content, which ranges from 2.3 - 16.8 %, while due to protein content, the variations are much smaller and range from 14 - 18% (Vladau et al., 2008; Trbović et al., 2009; Čirković et al., 2011). However, the protein content of fish meat is not strongly influenced by external nutrition because it mostly depends on internal factors such as the fish species and size of the fish.

The differences in ash content in the common carp meat from both environments are quite minimal and were not statistically significant.

The energy value (EV) of common carp meat was calculated according to the formula. The significantly higher energy value is established in the common carp meat from aquaculture (507.817 kJ/100 gr) compared to the meat from open waters (285.706 kJ/100 gr), as a result of significantly higher values of fat and protein content in aquaculture common carp.

According to the fat content, fish are classified into: lean fish (fat content of less than 2%), fish with small fat content (2 - 4 %), moderate fatty fish (4 - 8 %) and fatty fish (more than 8% fat) (Ackman, 1989). According to the received fat content in our study, common carp from aquaculture is being classified as moderate fatty fish (4.23 % fat), while common carp from open waters as fish with small fat content (2.92 %). These differences probably stem from the location of the fish samples, the season, the nutritional condition of the fish, the conditions in the aquatic environment, the fish size and age, etc.

4. Conclusions

Considering the results of the microbiological analysis for the total number of microorganisms on fish skin, the average value in common carp of aquaculture was 4.26 log CFU/cm^2, while in common carp of open water, 3.66 log CFU/cm^2.

The results obtained during the examination of the chemical composition of common carp meat from aquaculture and open waters determined the mean value of 71.27% water, 19.98% protein, 4.23% fat and 0.98% ash, as well as, 76.03 % water, 10.02 % protein, 2.92 % fat and 1.06 % minerals, respectively.

The differences in the values of certain chemical parameters shown that there is a significant difference ($p>0.05$) between the protein content of common carp from aquaculture and open waters, respectively.

A significant difference ($p<0.05$) is also considered between the fat of common carp from aquaculture and open waters, respectively.

There is a correlation between the fat content of fish meat and the percentage of water in muscle tissue, and the relationship between these indicators depends on the ambient conditions of the water ecosystem.

Regarding the fat percentage, i.e. 4.23% (common carp from aquaculture) and 2.92% (common carp from open waters), the examined carp samples belong to the group of fish with a small amount of fat (open water common carp) and medium fat fish (aquaculture common carp).

The results obtained for the energy value of fish meat indicate that the energy of the common carp meat from aquaculture (507.817 kJ/100g) is significantly higher compared to the one from open waters (285.706 kJ/100 g). These differences are due to the higher fat and protein content of aquaculture carp.

Feeding, dietary supplements, food conversion as well as food supplements increase (or restore) the growth rate and this is generally related to the increase of fat content. Other factors (temperature, steroid supplements) indirectly stimulate the diet resulting in increased fat content. This is very important because the intake of high - quality food to stimulate growth and reduce the breeding time in aquaculture production results in an increase in the fat content of fish meat.

In fish from aquaculture, the differences in chemical composition are less variable since in these systems the breeding factors can be controlled. The results suggest that the chemical composition of the fish may vary greatly during the catch season. This is due to physiological factors and environmental changes, i.e. spawning, migration, starvation or overeating.

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UTILIZATION OF DRIED PARSLEY LEAVES (*PETROSELINUM CRISPUMM*) AND THEIR ESSENTIAL OIL FOR EXTENDING SHELF LIFE OF BEEF BURGER

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ABSTRACT

In an attempt to raise the quality and extend shelf life of beef burger, parsley powder or its essential oil were added to the formula. Three different formulas of beef burgers were prepared. The first one was formulated with 0.5% dried parsley herb ,the second formulated with600ppm of parsley essential oil , and 3rd was free from additions (control).The results showed that the initial pH value of control beef burgers(C) and samples treaded with parsley powder or oil (P or O) was the same(5.8), but, after 8days of storage at 4ºC,the pH value significantly increased in all samples .the highest increase was recorded for sample(C), while sample(O) had the lowest increase. As for the, TBA values the data showed that addition of parsley oil to burger formula(O) significantly (P≤0.05) lowered the TBA value than control sample(C) at zero time of storage .The lowest significant increase in TBA value of all treatments, after8 days of storage at 4ºC, was recorded for(O)sample. The results also indicated that beef burger sample (O), at zero time ,had the highest significantly cooking loss and the lowest cooking yield than other burger samples. Simultaneously, beef burger sample (P) had the significant lower cooking loss and the higher cooking yield than other beef burger samples. After cold storage of all burger samples at 4ºC for 8 days, cooking loss values of all burger treatment were significantly increased. Control sample(C) had the highest significant increase in cooking loss. At the same time, burger sample (P) had the significantly higher cooking yield than other burger samples at the end of storage period. Moreover, beef burgers containing parsley oil or parsley powder have extended the lag phase period to 4 days while control samples have lower lag phase period 2 days for total Bacterial Count (TBC) and total Psychrotrophic bacterial Count. Same data indicated that parsley oil has the highest inhibitory effect against TBC and psychrotrophs count at zero time and till the end of cold storage. Meanwhile, parsley powder recorded a lower inhibitory activity than parsley oils against TBC and psychrotrophs count at zero time and till the end of storage. The results also observed that the total of the three color spectral readings L*, a* and b* values of fresh beef burger are affected by formulation with parsley essential oil or powder. Sensory evaluation showed that beef burger formulated immediately with parsley oil was superior in all quality attributes compared to samples formulated with or without powdered parsley. All recorded sensory score characteristics of control samples, at the 8th days, were rejected. While samples formulated with parsley oil or powder were acceptable till the8th day, with obvious superiority of beef burger formulated with parsley oil.

1.Introduction

Elsharawy *et al*.(2018) reported that Enhancement of food safety is the major interest by increasing interest in natural preservatives, which has antioxidant, antimicrobial properties and more healthy specially in meat products which is highly susceptible to microbial growth, which can cause its spoilage and contributes to food borne diseases in human, resulting in serious health problems. Parsley (*Petroselinum crispum*) is a species of *Petroselinum* in the family *Apiaceae*, originated in Europe and the Mediterranean region (Italy, Greece, Egypt, Algeria, and Tunisia), and widely cultivated as a herb, a spice, and a vegetable.

Brkovic *et al.* (2006) reported that plants from family *Apiaceae* are commonly used as food, flavoring agents and for medical purposes and are also known as nutraceutical plants. Based on many studies, it seems that several species in this family are good sources for phytochemicals with potent antimicrobial and anti inflammatory properties.

Karimi *et al.* (2014) reported that parsley essential oil shown a variable degree of antimicrobial activity on different microorganisms. Therefore using parsley essential oil as antimicrobial additive in food may be useful and alternative medical therapy for microorganisms which may resist customary treatment. This will suggest a great help in facing appearance spread of bacteria.

Alsaiqali *et al.*(2016) stated that meat and meat products such as beef burger are subject to spoilage either by microbes or by fat oxidation. Accordingly, it is very important to protect them from spoilage by adding preservatives, especially natural preservatives to extend their shelf life and to improve their characteristics.

Alsaiqali *et al. (*2016) found that parsley oil had a great reduction effect on the numbers of TBC, *S. enterica, E. coli, S. aureus* and *P. aeruginosa* during the first 2

The objective of our study was to investigate the effect of formulate beef burger with parsley dry herb or its essential oil on its quality

2.Materials and methods

2.1.Materials

Ten kg of recently dried parsley herb (*Petroselinum crispum*) was purchased from Medicinal and Aromatic plants Research Department, Horticulture Research Institute, Horticultural Research Center, Giza, Egypt.

Fifty ml. of parsley leaves essential oil were obtained as a gift from Greetco Co. Abo Elnomros, Giza, Egypt.

2.2.Methods

2.2.1. Manufacturing of beef burgers:

Three different formulas of beef burgers were prepared. The first one was formulated with 0.5% dried parsley herb, the second was formulated with600ppm of parsley essential oil, and 3rd was free from additions (control). The beef burger formulas were prepared as described by Emrick *et al.* (2005) and compatible with EOS (2005) as presented in Table (1). All ingredients were mixed together in a blender to ensure uniform distribution of added ingredients.

Table 1. Formulation of beef burgers with dried parsley herb and its essential oil

Ingredients	**Treatments %**		
	Control	**Dried Parsley herb**	**Parsley herb oil**
Minced meat	90	89.50	89.40
Salt	1.5	1.5	1.5
Milk powder	3	3	3
Spice mixture	2	2	2
Fresh onion	3.5	3.5	3.5
Parsley powder	-	0.5	-
Parsley oil	-	-	600mg/1kg meat

The burgers formulae were shaped manually using a patty maker (stainless steel model form) to obtain round discs of 10 cm in diameter and 0.5 cm thickness with average weight 50 gm. Burgers were packed in polyethylene bags and kept into laminated package and stored at 4ºC for 8 days.

2.2.2.Cooking of beef burgers

The beef burgers were cooked for 4min each side at 200ºC as mentioned by Ali *et al.* (2011)

2.2.3.Color of raw beef burgers

Color measurement of different prepared beef burgers with dried and oils of parsley. color was measured by Chroma meter (Konica Minolta, model CR 410, Japan) calibrated with a white plate and light trap supplied by the manufacturer. Color was expressed using the CIE L*, a*, and b* color system (CIE, 1976). A total of three spectral readings were taken for each sample. Lightness(L*) (dark (0) to light (100)), the redness (a*) values ((+) reddish to (-) greenish). The yellowness (b*) values (+)yellowish to (-) bluish) were estimated. According to CIE (Commission International de l'E´ Clairage) (1976). Official recommendations on uniform color spaces. Color difference equations and metric color terms, Suppl. No. 2. CIE Publication No. 15 Colourimetry, Paris.

Beef burger samples were analyzed in Cairo University Research Park (CURP), Faculty of Agriculture.

2.3.Microbiological analysis

The microbiological analysis of control sample and the two treated beef burger formulas (with dried parsley herb and its oil) includes the determination of total bacterial count and Psychrotrophic bacterial count.

2.3.1.Sample preparation

Ten grams of uncooked beef burger samples were mixed with 90ml of sterile saline solution (8.5gm NaCl/1Ldistilled water) in a blender, under aseptic conditions, to give 1/10 dilution .Serial dilutions were prepared to be used for counting total bacterial count and psychrotrophic bacterial count, immediately after each storage period at Zero time (fresh), 2,4and 8days at 4ºC according to American Public Health Association (APHA, 1992).

2.3.2.Total Bacterial Count (TBC) and psychrotrophic bacterial count

The total bacterial and psychrotrophic bacterial counts were determined using plate count agar medium according to the procedure described by (APHA,1992). The plates were incubated at 37ºC for 48h, and7ºC for10 days for total bacterial count and for psychrotrophic bacterial count, respectively. The results were expressed as colony forming units per gram (CFU/g).

2.4.Chemical analysis of the beef burgers

2.4.1.Thiobarbituric acid (TBA)

Extraction procedure:

Twenty grams of sample were homogenized with 100 mg of butylated hydrxytoluene (BHT) and 100ml of 7.5 % trichloroacetic acid (TCA) solution, mixed for one minute in solvents blender and filtered through a filter paper (Whatman No.1),then 5 ml of TBA reagent (0.02 M TBA,0.2896 g/100ml glacial acetic acid) were added to 5 ml of filtrate in test tubes with screw caps. They were placed for 40 min. in a boiling water bath. Absorbance was read at 538 nm after cooling in tap water, the original TCA extract was used as a blank,(Vyncke,1970).The TBA values were calculated according to(Zeb and Ullah , 2016).

Y=1.4167X+0.0785

Where 'x' is absorbance

2.4.2.Measurement of pH value

Beef burger samples (10 g) was homogenized with 50 mL deionized water for 1 min. The pH value was measured at room temperature using a digital pH meter3305, Jenway (Gahruie *et al.,* 2017)

2.4.3.Sensory evaluation

Cooked beef burger samples were sensory evaluated immediately after cooking. All cooked beef burger samples were cut to small slices and coded with random numbers as described by AMSA (1995). Sensory evaluation was performed by 12 panel from postgraduate students and staff of the faculty of Agriculture Cairo university. Evaluated five parameters as odor, color, taste, texture and over all acceptability using a 9-point hedonic scale, as follows (9 Excellent, 8 Very good, 7 Good, 6 Acceptable, 5 Poor). The mean values of the obtained results from sensory evaluation were statistically analyzed.

2.5. Statistical analysis

Data were statistically analyzed one way by analysis of variance, ANOVA, (Rao and Blane, 1985). Data were presented as means of 3 experiments ±SD unless otherwise stated. All microbial data were transformed to logarithm before analysis.

3.Results and discussion

3.1.Physico-chemical properties of beef burgers

Data in Table (2) show the physico-chemical properties of beef burgers as affected by addition of parsley oil (600ppm) or its powders (0.5%). From these results observe the changes occurred in pH values of beef burgers during storage at 4ºC for 8days. The initial pH value of control beef burgers(C) and samples treaded with parsley powder or oil (P and O) was the same(5.8), but, after 8days of storage at 4ºC,the pH value significantly increased to 6.3 for sample(C), 6.2 and6.1 for samples(P),(O), respectively. Also, Elsharawy (2018) reported that the addition of oils or powder of parsley did not affected the pH values of meat products.

During cold storage at 4ºC for 8days, the pH values of burgers (control and with parsley oil or powder) were increased significantly with increasing storage time. The highest increase in pH value (6.3) was recorded for control sample while, sample formulated with parsley oil had the lowest increased pH value (6.1).The Increase in pH value could be attributed to the formation of volatile basic nitrogen components as affected by biochemical changes under low temperature These results are in agreement with those obtained by *Alsaiqali et al. (2016)*.

The increment of pH value of control burger samples might be due to the effect of microbial growth which may cause protein hydrolysis and release of nitrogenous compounds that increase the pH value of meat.

Also, the data in Table (2) indicate about the TBA values -the oxidative rancidity- of the samples. The limit of acceptance for TBA is proposed as 0.9 mg as malondialdehyde/kg raw minced beef, according to EOS (2005)". The data showed that the TBA values of (O) sample was significantly ($P \leq 0.05$) lower than control sample(C) at zero time of storage. While the value of TBA determined in samples contained parsley herb was significantly insignificant from the control.

During 8 days of storage, the highest increase in TBA value was observed for control samples at the end of storage time, while samples contained parsley oil and parsley powder had lower TBA values. It is noticeable that greatest stability of burger samples was recorded for parsley oil sample(O) over parsley powder sample, where as they recorded to 0.36 or 0.38 mg malondialdehyde/kg sample, respectively, after 8days storage at 4ºC.IN addition, the increase in the value of TBA of the sample (O) was significantly less than the increase in control(C). After the same time of storage the TBA value of control samples was twofold the beginning sample (it reached to 0.5mg malondialdehyde/kg sample). Our results are agreements with those obtained by Abd El-Qader *et al*.(2004)., Kassem *et al*. (2011) and Alsaiqali *et al.(*2016).

Table 2. Effect of addition of parsley oil (600ppm) or dried leaves powder (0.5%) on pH, TBA, Cooking loss and Cooking yield of beef burger during storage at 4°C for 8 days.

	Treatments	Storage time (day)			
		0	2	4	8
pH	Control (C)	$5.8^{Ac}\pm0.00$	$6.0^{Ab}\pm0.07$	$6.2^{Aa}\pm0.00$	$6.3^{Aa}\pm0.07$
	Powder(P)	$5.8^{Ab}\pm0.07$	$5.9^{Ab}\pm0.07$	$6.1^{ABa}\pm0.07$	$6.2^{Aa}\pm0.07$
	Oil(O)	$5.8^{Ab}\pm0.07$	$5.9^{Ab}\pm0.07$	$6.0^{Ba}\pm0.00$	$6.1^{Aa}\pm0.00$
TBA (mg malondialdehyde/kg)	Control (C)	$0.23^{Ac}\pm0.01$	$0.28^{Ac}\pm0.00$	$0.38^{Ab}\pm0.04$	$0.50^{Aa}\pm0.01$
	Powder(P)	$0.22^{ABd}\pm0.00$	$0.25^{Bc}\pm0.00$	$0.29^{ABb}\pm0.00$	$0.38^{ABa}\pm0.00$
	Oil(O)	$0.20^{Bb}\pm0.00$	$0.23^{Bb}\pm.01$	$0.27^{Bb}\pm0.00$	$0.36^{Ba}\pm0.05$
Cooking loss (%)	Control (C)	$9.77^{Ac}\pm0.22$	$10.52^{ABc}\pm0.41$	$14.02^{Ab}\pm0.52$	$15.39^{Aa}\pm0.40$
	Powder(P)	$8.44^{Bb}\pm0.46$	$9.00^{Bb}\pm0.62$	$9.60^{Bb}\pm0.60$	$12.13^{Ca}\pm0.30$
	Oil(O)	$10.48^{Ad}\pm0.15$	$11.53^{Ac}\pm0.64$	$12.80^{Ab}\pm0.16$	$13.82^{Ba}\pm0.04$
Cooking yield(%)	Control (C)	$90.24^{Ba}\pm0.22$	$89.48^{ABa}\pm0.41$	$85.99^{Bb}\pm0.52$	$84.62^{Cc}\pm0.40$
	Powder(P)	$91.57^{Aa}\pm0.46$	$91.01^{Ab}\pm0.62$	$90.41^{Ac}\pm0.60$	$87.87^{Ad}\pm0.30$
	Oil(O)	$89.53^{Ba}\pm0.15$	$88.48^{Ba}\pm0.64$	$87.20^{Ba}\pm0.16$	$86.19^{Bb}\pm0.04$

Any two means within the same column have different capital letters, and any two means within the same row have different small letters are significantly different at $P<0.05$.
C: Control, P: Parsley Powder, O:Parsley Oil. SD, standard deviation of group means.

3.2.Cooking loss

The aim of cooking is to kill most of microorganism, inhibit toxic substance, augment digestibility and improve organoleptic properties of meat products. But cooking causes some loss in weighting (cooking loss) which affects the yield of cooked meat. Results in Table (2) showed the cooking loss and cooking yield of different studied treatments during storage at 4°C. It could be observed that, beef burger sample treated with parsley oil(O),at zero time ,had the significantly higher cooking loss and the lower cooking yield than other beef burger samples, simultaneously, beef burger sample treated with parsley powder(P) had the significantly lower cooking loss and the higher cooking yield than other beef burger samples. After storage of all burger samples at 4°C for 8 days, cooking loss values of all burger treatment were significantly increased .Control sample(C) had the highest significant increase in cooking loss .At the same time, burger sample (P) had the significantly higher cooking yield than other beef burger samples at the end of storage period.

The significant decrease in cooking loss% and significant increase of cooking yield % in samples (P) than the (C)samples could be related to the Absorption capacity of parsley powder. On the other hand, cooking loss% of samples (O) increased and cooking yield % decreased than (C) samples, this could be attributed to the high loss of fat and oil during cooking as reported by (kim *et al.,* 2016).

The higher cooking loss and lower cooking yield of(C) samples than the other samples could be attributed to the excessive fat separation and water release that occurred from breaking the emulsion during cooking (kim *et al*.,2016).

By increasing storage time at 4°C, the cooking loss increased and cooking yield decreased of all studied samples formulas this may be occurring due to the decreasing in water holding capacity, moisture retention and fat retention of these treatments during storage. Similar trends in cooking loss were observed by Abd El-Qader (2004) and Ali (2008) who found that cooking loss of chicken and beef burger increased as the period of storage increased. Also, Abolgasem (2011) and Hamza (2011) mentioned that cooking loss of beef burger significantly increased by advancement of storage time. Moreover, Hussein (2015)

reported that high cooking loss of fat burger might be attributed to the excessive fat separation during cooking.

Effect of addition of parsley powdered leaves or their essential oil to beef burger formula on total bacterial and Psychrotrophic counts during cold storage.

Table 3. Effect of addition of parsley powdered leaves or their essential oil to beef burger formula on total bacterial and psychrotrophic counts during cold storage (4ºC for 8days).

	Treatments	Storage time (day)			
		0	2	4	8
TBC (cfu/g)	**C**	$3.8x10^{4Ad}$	$5.8x10^{4Ac}$	$3.5x10^{5Ab}$	$8.0x10^{5Aa}$
	P	$3.5x10^{4Bd}$	$4.8x10^{4Bc}$	$6.5x10^{4Bb}$	$5.0x10^{5Ba}$
	O	$3.2x10^{4Cd}$	$4.4x10^{4Cc}$	$5.4x10^{4Cb}$	$3.2x10^{5Ca}$
Psychrotrophs (cfu/g)	**C**	$4.5x10^{4Ad}$	$8.0x10^{4Ac}$	$3.8x10^{5Ab}$	$7.4x10^{5Aa}$
	P	$4.0x10^{4Bd}$	$6.3x10^{4Bc}$	$9.5x10^{4Bb}$	$4.3x10^{5Ba}$
	O	$3.8x10^{4Cd}$	$5.0x10^{4Cc}$	$8.0x10^{4Cb}$	$3.7x10^{5Ca}$

Any two means within the same column have different capital letters and any two means within the same row have different small letters are significantly different at $P<0.05$.

C: Control, P: Parsley Powder, O: Parsley Oil

Table (3) Shows the effect of formulate beef burger with parsley powdered leaves (0.5%) or their essential oil (600ppmon total bacterial count and total psychrotrophic bacterial count during storage at 4ºC for 8days. Beef burgers containing parsley oil or parsley powder have extended the lag phase period to 4 days while control samples have lower lag phase period 2 days for total Bacterial Count (TBC) and total Psychrotrophic bacterial Count. Same data indicated that parsley oil has the highest inhibitory effect against TBC and Psychrotrophs count at zero time and till the end of cold storage. Meanwhile, parsley powder recorded lower inhibitory activity than parsley oils against TBC and psychrotrophs count at zero time and till the end of storage. Similar results were recorded by Alsaiqali *et al*.,(2016) who also noticed the effect of essential oils on burger after few days of cold storage.

The reported reduction of TBC rates might referred to the presence of some antibacterial compounds in parsley oils or powder as, (flavonoids) which affect most micro-organisms and the bacteriostatic compounds as phenols. Similar results reported by Wong and Kitls (2006); Ashour *et al*.(2014), Farah *et al*.(2015) and Alsaiqali *et al*.(2016) who observed that the extract of dill and parsley had significant reduction of TBC and dill was more effective than parsley extract. Moreover Alsaiqlali *et al*.(2016) concluded that parsley extract had a strong antimicrobial effect against TBC, *E.coli* ,*S.aureus* during the first storage period however this effect reduced by time. We recommended to using parsley oils and powder as a safe and natural antimicrobial meat additive to prolong shelf life of meat burger.

Total bacterial counts and Psychrotrophic bacterial count in control samples was significantly higher ($P\leq0.5$) than the samples containing parsley oil(600ppm) and samples containing parsley powder (0.5%) at zero time of storage at 4ºC. These results have the same trend with the results obtained by Elsharawy (2018).

3.3.Color measurement

Color measurements can be used as an indirect way to estimate undesirable change in food products, since it is a simple and faster method than chemical analysis Gahruie *et al.* (2017)

Table 4. Changes in beef burger color due to formulation with parsley oil or powder

Samples	L*	a*	b*
Control	$44.0^{B}\pm0.26$	$11.8^{A}\pm0.23$	$4.2^{B}\pm0.06$
Oil	$45.1^{A}\pm0.25$	$11.2^{B}\pm0.14$	$6.6^{A}\pm0.41$
Powder	$44.5^{B}\pm0.35$	$8.6^{C}\pm0.16$	$7.2^{A}\pm0.49$

Any two means have different capital letters at the same column are significantly different at $P<0.05$.
±= SD standard deviation of group mean.

Results in Table (4) showed that the lightness (L* value) of the burger samples ranged from 44.0 for control to 45.1 for samples with parsley oil and 44.5 for samples treated with parsley powder. The control samples and which treated with parsley powder had significantly lower L value when compared with sample treated with parsley oil. Also redness and yellowness (a*and b* value) ranged from 11.8 to 8.6 and 4.2 to 7.2 for control and samples treated with parsley powder, respectively. The burger samples which treaded with parsley powder had lower redness value than other treatments. On the other hand b* value (yellowness)was higher for samples treaded with parsley powder and parsley oil when compared with control. Results showed that the yellowness (b* value) of parsley powder was significantly higher than the control sample. These results are in agreement with Riel *et al.*(2017) who found that, the Internal contents of parsley extract as a nitrite source in mortadella sausages led to the rise in b* value of the sausage.

In general, it could be concluded that L*, a* and b* values of fresh beef burger are affected by formulation with parsley oil(600ppm) or powder (0.5%).

3.4.Sensory evaluation

Results reported in Table (5) observed the means values of sensory characteristics of beef burger formulated with parsley powder or its essential oil (odor, taste, texture, color and overall acceptability) immediately after formulation and during cold storage at 4°C for 8 days. The results showed that beef burger formulated with parsley oil was superior in all quality attributes compared to samples formulated with or without powdered parsley. Parsley oil samples got the highest significant scores of odor, color, taste and overall acceptability, but parsley powder samples got the highest ,insignificant, scores of texture when compared with samples formulated with parsley oil as shown in Table (5).These data declared that formulation of beef burger with parsley oil , and to a lower extent parsley powder, improve most of its quality attributes.

Table 5. Effect of parsley oil and parsley powder on sensory evaluation of beef burger during storage at 4°C for 8days.

Treatments	Storage (days)	Odor	Color	Taste	Texture	Overall acceptability
Control(C)	**0**	$8.0^{bcd}\pm0.60$	$8.5^{bc}\pm0.52$	$8.2^{cde}\pm0.58$	$8.1^{b}\pm0.29$	$8.1^{bc}\pm0.67$
	2	$7.9^{bcd}\pm0.58$	$8.3^{c}\pm0.49$	$8.0^{cde}\pm0.29$	$8.0^{b}\pm0.43$	$8.0^{bc}\pm0.43$
	4	$6.8^{e}\pm0.62$	$7.7^{d}\pm0.49$	$6.5^{f}\pm0.52$	$7.0^{c}\pm0.74$	$7.0^{d}\pm0.60$
	8	$5.0^{f}\pm0.74$	$5.5^{f}\pm0.52$	$4.3^{h}\pm0.78$	$6.6^{c}\pm0.51$	$4.8^{f}\pm0.72$

Powder(P)	**0**	$8.3^{b}\pm0.65$	$7.5^{d}\pm0.90$	$8.6^{bc}\pm0.67$	$9.2^{a}\pm0.83$	$8.5^{b}\pm0.67$
	2	$8.1^{bc}\pm0.67$	$7.4^{d}\pm0.79$	$8.1^{bcd}\pm0.52$	$9.0^{a}\pm0.79$	$8.4^{b}\pm0.67$
	4	$7.7^{cd}\pm0.49$	$7.4^{d}\pm0.79$	$7.7^{e}\pm0.65$	$8.1^{b}\pm0.79$	$7.8^{c}\pm0.39$
	8	$6.8^{e}\pm0.45$	$6.0^{ef}\pm0.60$	$5.7^{g}\pm0.78$	$7.0^{c}\pm0.43$	$5.9^{e}\pm0.67$
Oil(O)	**0**	$9.3^{a}\pm0.62$	$9.2^{a}\pm0.58$	$9.2^{a}\pm0.72$	$9.1^{a}\pm0.67$	$9.3^{a}\pm0.65$
	2	$9.2^{a}\pm0.58$	$9.0^{ab}\pm0.43$	$9.0^{ab}\pm0.43$	$9.1^{a}\pm0.60$	$9.1^{a}\pm0.51$
	4	$8.3^{b}\pm0.49$	$8.4^{c}\pm0.51$	$8.0^{de}\pm0.43$	$8.0^{b}\pm0.60$	$8.0^{bc}\pm0.60$
	8	$7.5^{d}\pm0.5.2$	$6.5^{e}\pm0.52$	$6.0^{fg}\pm0.74$	$6.6^{c}\pm0.51$	$6.1^{e}\pm0.67$

Any two means have different superscript letters within the same column are significantly different at $p<0.05$.
±= SD, standard deviation of group means.
C=control, P=powder 0.5% and O=oil 600ppm.
9=Excellent 8= Very good 7= Good 6= Acceptable 5= Poor

The sensory characteristics of all burger samples showed continuous decrease along storage period and the lowest quality attributes were reported on the 8th day of storage for control sample.

All recorded sensory score characteristics of control samples, at the 8th days, were rejected. While samples formulated with parsley oil or powder were acceptable till the8th day, with obvious superiority of beef burger formulated with parsley oil.

It is worth to mention that significant improvements of odor, taste and texture of burger samples which treated with parsley oil or powder could be attributed to the aromatic effect of parsley oil or powder as reported by Mishra and Dubey (1994), Lawless (1995). Therefore, much attention in recent years has been focused on the use of extracts from herbs and spices to improve sensory characteristics, retard lipid oxidation and extended the shelf life of meat products (Arorakour,1999, Gulluce *et al*.,2003 and Lagouri and Nisteropoulou, 2009).

4.Conclusions

From the obtained results it could be concluded that addition of parsley oils or powder to beef burger formula at concentration of 600 mg/kg or 0.5% respectively, not only to minimize lipid oxidation but also to improve sensory characteristics and enhanced the wholesomeness of the product during refrigeration storage due to lower TBA value and microbial load .We recommended to use parsley oil or powder as a safe and natural antimicrobial meat additive to prolong shelf life of beef burger and, above all, the improvement of most sensory properties.

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VERIFICATION OF THE TRACEABILITY MODEL OF AUTOCHTHONOUS POULTRY BREEDS

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ABSTRACT

Microsatellite markers are reliable and cheap method for studying diversity among animal breeds. They are widely used for separation of related animal breeds on genetic level. When used in food industry, they have great potential to be used for authentication of animal food products. We are aiming to explore the variability of alleles in selected markers in modeled F1 generation of Slovak breeds of chicken. We want to compare, if previously proposed traceability model is relevant for next generation of chickens or it is limited to one, parental, generation. Our analysis was based on 7 selected microsatellite markers. We modeled genotypes of 42 F1 generation individuals of Oravka tawny and 42 of Oravka white, derived from 1 rooster and 7 chickens from each breed. In our study, we used PCoA analysis and neighbor joining (NJ) analysis. With usage of both analyzes, we proved, that both generations are unique and genetic distance between individuals of different color breed are wide enough. We proved, that we only need to genotype the parental generation of both Oravka chicken tawny and white breeds. After creating F1 generation, we are reliably able to separate those populations. There is no need to genotype whole F1 generation. This provide huge financial benefits. Furthermore, we are able to trace and authenticate whole F1 production generation.

1.Introduction

Providing the food and economic income, production of poultry serves an important role, especially for small scale farmers. Despite the importance of local breeds of poultry, their populations have been decreasing in recent years. Some breeds are even becoming endangered and threated by extinction. This is because of low performance of local chickens compared to highly specialized lines that have high performance, mixed rearing with other breeds, and lack of suitable conservation strategies to protect local chickens. Decreasing numbers of local chicken may be connected to the loss of valuable genetic variability and unique traits and characteristics. The convention on biological diversity has put the need to conserve farm animal genetic diversity on the agenda. Regarding to the farm animal diversity conservation, a unified approach accounting for two main roads to conservation has been established. This includes the prevention of breed extinction, and management of within breed genetic variability with the main objective of controlling genetic drift (Simianer, 2005). In

last few years, the goal of preserving genetic diversity has become more and more important. The very beginning of the commercial breeding of various poultry species took place since the middle of 20th century. Since that, industrial breeding of chicken was based on rather small circle of highly specialized lines, which originated from small number of world's most common specialized breeds. Due to this, large number of combined breeds of chickens are on the brink of extinction by long-term breeding. This is indicated by the data on the increasing rates of exhaustion of the gene pool as a result of the crowding out local breeds by transnational commercial breeds (Abdelaziz et al., 2019). Since the start of commercial poultry breeding, chicken genetic diversity has become partitioned among relatively few highly specialized lines. As a result of this, many dual-purpose breeds, originated from centuries-long domestication and breeding, are now in the danger of perishing. However, these older breeds pose a resource of genes valuable for the future breeding activities and research purposes. Therefore, it is necessary to assess the diversity at the molecular level, in a wide range of chicken populations, including commercial lines, traditional breeds, experimental lines, and the red jungle fowl, in order to provide recommendations regarding to the future management or conservation of chicken biodiversity. Even though the decision on conservation of population 's gene pool must be based on various sources of information, including specific traits interesting for breeding, molecular markers has a potential to be helpful and important initial guide (Hillel et al., 2003). Biodiversity conservation is a topic of interest and domestic animal diversity is an esential component (FAO, 2011). Although scientists are focused on genetic resources of all farm animals, the conservation of poultry has attracted increasing attention for years now (Tadano et al., 2013). In comparison to fast-growing broilers, older, native chicken breeds and their hybrids have lower gain of weight, smaller proportions of breast muscle in the carcass. However, their meat has many quality characteristics, which are highly valued by modern day consumers (Sokołowicz et al., 2016). Oravka is a dual-purpose breed of chicken, originated from Slovakia region of Orava. Oravka was bred with the purpose of being able to adapt to harsh conditions of their native environment. This breed is also the only one with the status of native chicken in Slovakia. Development of Oravka chicken started in 1950s under the guidance of the Research Institute for Poultry. First stage was a combinatorial crossing of regional breeds with breeds of Rhode Island Red, New Hampshire and Wyandotte White (Chmelničná, 2004). The goal was to develop a breed, which can be kept in free range, and be suitable for harsh climatic conditions of northern Slovakia. It was adapted for egg and meat production. In 1990 was recognized as an independent breed (Hanusová et al., 2017). In the animal production sector, microsatellite markers have been used for longer than a decade for characterization and conservation of livestock biodiversity, as well as for traceability of food products. Genotyping standards are currently used for livestock animals. Those are standardized SNP panels, which allows the characterization of tens, even hundreds of thousand markers per sample. Microsatellite markers still represents a useful tool used for characterization of animal breeds mostly because of their low cost and easy implementation of genotyping protocols. FAO published recommendations for standardized sets of microsatellite loci to be used for studying diversity in major livestock species in order to make possible the comparison of results across different research projects (Abdurakhmonov, 2016). When it comes to individual identification and parentage assessment, genetic markers are important resources. Short tandem repeats (STRs) have been the traditional DNA markers of choice in many species. However, advantages in technologies show that nucleotide polymorphisms (SNPs) are becoming an attractive alternative, especially in recent years. SNPs can be highly multiplexed and automatically scored, which allows an easier standardization and sharing among different

laboratories. The domestic horse currently uses STR based DNA typing. (Seo et al., 2013). Microsatellite markers have many applications in molecular studies. They are proven to be most accurate and efficient tools for studying genetic diversities and relationships (Dávila et al., 2009) connections among populations and genetic fitness of whole populations, including paternity determination (Witzenberger and Hochkirch, 2011). Since the PCR technologies became commonly available, it has been easy to amplify DNA isolated from both plants and animals. Microsatellite has short repeat units and it is a good example of nuclear DNA markers (Rosenbom et al., 2015) that has found its wide application in molecular studies. Comparing to other markers, the greatest advantages of

microsatellite markers are their high variability and distribution (Yilmaz et al., 2015). Extent of diversity in livestock populations across the globe has been gained through the usage of microsatellite markers (Granevitze et al., 2007). Given the fact that they can easily show the information essential for creating long term breeding plans, designing of breeding programs planning conservation strategies, traceability and in parentage verifications. In recent years, selection of microsatellite markers has been based on single criterion called polymorphism information content. For determination of this parameter, researchers have been using the formula suggested by

With the proof that we are able to separate populations of Oravka tawny and white, it is expected that we will be able to do so even with F1 generations of observed and genotyped chickens. Despite being genetically closest, Oravka tawny and white could be separated. With low variability in selected markers within one population, there is a low chance for the first generation of selected chickens to close the genetic distance between them.

Botstein et al. (1980) and when PIC value of a marker equals the threshold value of 0.50 or above, such marker is called to be informative (Olowofeso et al., 2016).

2.Materials and methods

For the research, we used genotyped individuals from work of Belej et al., (2019). We randomly selected one rooster and 7 hens of Oravka tawny (Table 1.) to simulate the breeding conditions in farm breeding process. We modeled 6 offspring from the rooster and each hen for total 42 F1 generation individuals. The same process was used with Oravka white population. The final step was comparing those populations to visualize genetic distance between F1 populations and parent individuals.

2.1 Statistical analysis

To create a random combination of alleles of modeled offsprings Microsoft Excel was used. To calculate the genetic distance between observed individuals, we used Microsoft Excel add-on program GenAlEx 6.5. Principal coordinates analysis (PCoA) was executed in GenAlEx 6.5 as well.

RStudio 3.6.0 ("Planting of a Tree") with additional adegenet and ape libraries software was used to perform and visualize the neighbor joining (NJ).

Table 1. Genotypes of parental generation

Sample	**LEI0254**		**MCW0034**		**LEI0192**		**LEI0166**		**MCW0069**		**LEI0234**		**LEI0228**	
Oravka tawny														
PAR1	86	86	219	221	289	293	348	348	155	159	292	292	162	174
PAR2	86	86	221	221	253	289	348	348	155	155	288	288	162	224
PAR3	86	86	221	221	269	293	348	348	155	163	288	292	162	162
PAR4	86	86	221	221	289	293	348	348	159	161	292	308	232	232
PAR5	90	90	221	233	269	297	348	348	161	165	292	292	162	174
PAR6	86	86	219	221	269	289	348	348	159	161	288	296	228	228

PAR7	86	86	233	233	289	289	348	348	159	163	292	308	164	228
PAR8	86	86	221	221	289	293	348	348	159	159	292	308	224	224
Oravka white														
WHP1	86	86	223	231	253	253	344	344	159	161	212	304	162	208
WHP2	86	86	221	223	253	253	344	352	161	161	212	260	208	208
WHP3	86	86	223	231	253	253	344	352	159	163	300	304	162	204
WHP4	90	90	229	229	253	253	344	348	155	165	212	212	228	232
WHP5	90	90	231	231	253	293	348	352	155	161	212	216	228	232
WHP6	86	86	227	229	289	289	348	348	161	161	300	312	162	162
WHP7	86	86	229	229	301	301	356	356	161	173	212	212	162	204
WHP8	86	86	221	223	253	253	344	352	161	161	212	260	208	208

3.Results and discussions

The concept of traceability throughout the food supply chain is recognised within the European Union with the regulation (EC) No. 178/2002, in which traceability is defined as the ability to trace and to follow food, feed and ingredients through all stages of production, processing and distribution. As a consequence, traceability requires systems for animal identification and registration and for labelling animal products, in order to ensure a link between the animal and the meat produced from it (Ammendrup and Fussel, 2001; Caporale et al., 2001).

Microsatellite markers had been widely investigated for many applications such as genetic identification, assessment of parentage, breed assignment tests and traceability (Dalvit et al., 2007; Rosa et al., 2013; Tolone et al., 2012; Sardina et al., 2015). Microsatellite markers have been recommended as the marker of choice for biodiversity studies (FAO, 2011). They are regarded as the most convenient tool for determination of heterozygosity and genetic distances. As they are numerous and randomly distributed throughout the chicken genome, they show a higher degree of polymorphisms, follow codominant inheritance and are ideal for deciphering genetic variability (Zhou et al., 2008; Abebe, et al., 2015). A prerequisite for the development of efficient SNP-based identification systems is the description of a minimal set with sufficient power to uniquely identify individuals and their parents in a variety of popular breeds and crossbred populations (Heaton et al., 2002), even though the information content in the SNP set may vary significantly between populations (Krawczak, 1999, Fernández et al. 2013).

As mentioned, our analysis was based od 7 microsatellite markers. The variability of alleles within the populations of selected chicken breeds is low. Chickens used in our study were genotyped by authors Belej et al., (2019). Genetic structure of selected chicken individuals is listed in Table 1. First individual in every sample column is a rooster, other 7 are hens. DNA based methods, with the use of microsatellites markers, are powerful tools already fully established in parentage typing, individual assignment (Jobling and Gill, 2004), population genetics (Moioli et al., 2001) and evaluation of genetic resources (Pariset et al., 2003); moreover, microsatellite based tests have been proposed for individual breed identification (Ciampolini et al., 2000). Recently, DNA identification techniques have also been proposed in the field of meat traceability to implement conventional animal identification. (Cunningham and Meghen, 2001; Stanford et al., 2001). These methods foresee that a meat sample, taken at any point in the retail chain, is analyzed by a DNA test, and the obtained fingerprint is compared with the DNA fingerprint of the animal which is thought to have given that meat. The animal DNA profile is obtained from tissue samples collected either from the living animal, or during slaughter, and then stored.

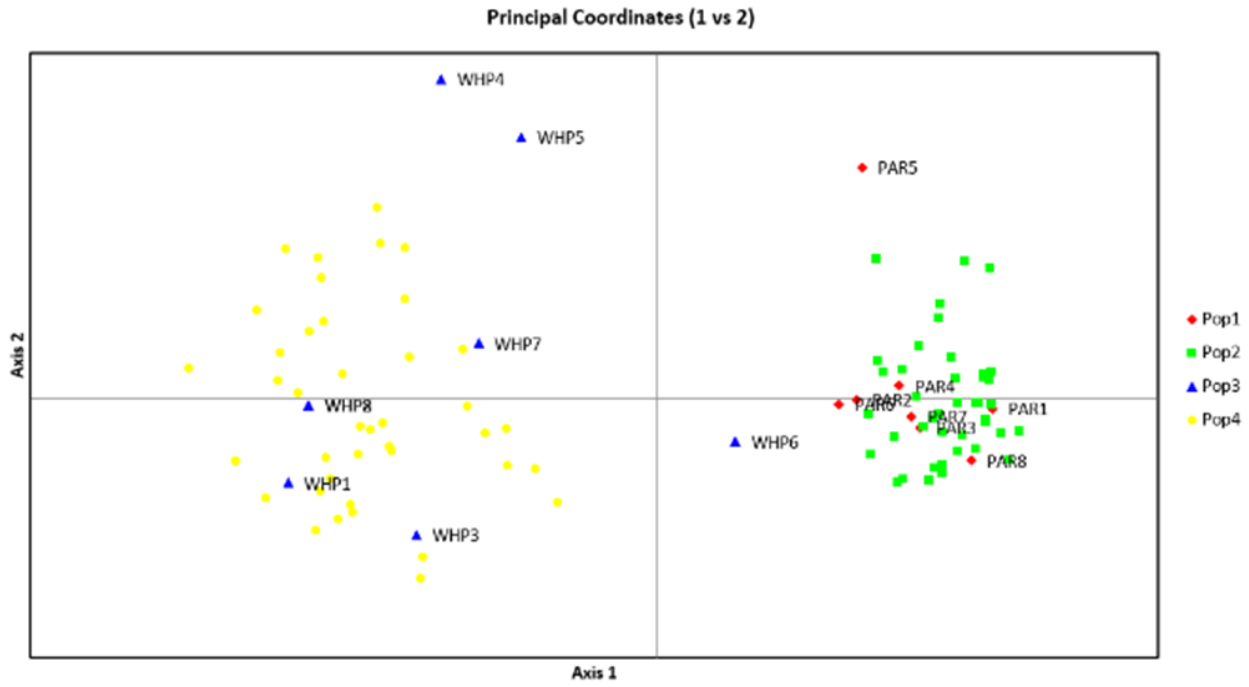

Figure 1. Scatter plot of PCoA analysis

Matching between the alleles of the two samples provide an information whether the declared origin of the meat is correct or not (Orrú et al. 2006). Microsatellites are considered to be the most variable types of DNA sequence in the genome. In contrast to unique DNA, microsatellite polymorphisms derive mainly from variability in length, rather than in the primary sequence. Moreover, genetic variation at many microsatellite loci is characterized by high heterozygosity and the presence of multiple alleles, which is in sharp contrast to unique DNA (Ellegren, 2004). Scatter plot of PCoA analysis shows that both parental generations and F1 generations are clearly separated. Percentage of variation explained by the first 3 axes are for axis 1: 40,38%, for axis 2: 7,57% and for axis 3: 6,42%. In the Figure 1 is showing the visualization of PCoA analysis using axis 1 and 2. Together, cumulative percentage of variation explained by the first 2 axes is 47,95%. Parental population of Oravka tawny is shown by red colour and marked as Pop1. It's the F1 population, Pop2 is in green colour. For Oravka white, blue Pop3 shows parental generation and yellow Pop4 modeled F1 generation. It is clearly visible that F1 generations are separated from each other. It means that not even on the level of the first generation, genetic distance between populations of Oravkas is not closing. The same is true for the ordering of genetic distances among populations assessed for different markers (Jorde et al., 2000). Therefore, it is reasonable to believe that the ordering breeds of diversity, among chicken, and distances seen here for microsatellites in DNA pools, would not be very different with using other genetic marker systems. The microsatellite loci used here were selected to be polymorphic for usage in gene mapping (Belej et al., 2019). Populations which do not fit with an ideal panmictic population are said to be structured (Barker, 1989). This structure is exhibited with a different pattern of frequencies and fixed alleles.

Pure breeds of livestock are the products of thousands of years of selection; they are closed populations and each of them shows a peculiar genetic structure (Orrú et al, 2006).

The algorithm of the neighbor joining (NJ) method is similar to that of the ST method, which objective is to construct the topology of a tree. On the contrary, the NJ method provides

not only the topology, but also the branch lengths of the final tree. Before discussing the algorithm of the present method, let us first define the term "neighbors." A pair of neighbors is a pair of OTUs connected through a single interior node in an unrooted, bifurcating tree (Saitou and Nei, 1987). As well as scatter plot, neighbor joining method proves, that our investigated F1 populations are segregated on genetic distance. The fan type neighbor-joining is a simple plot that connects the most genetically related individuals. In NJ plot - Figure 2 it is visible, that 2 different main branches were created. On those branches are individuals of both Oravkas populations, parental and F1 generations.

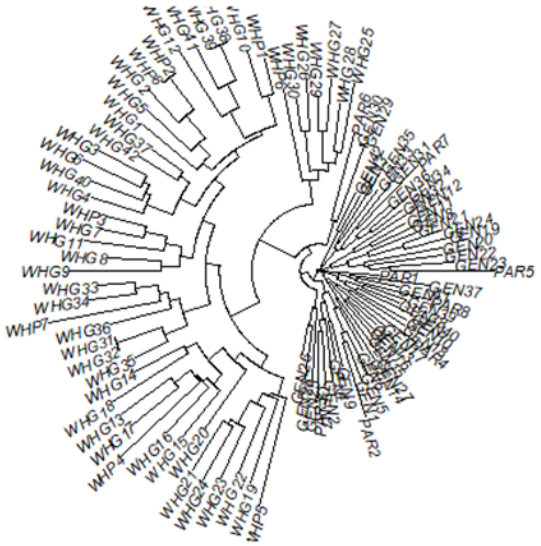

Figure 2. Vizualization of NJ analysis

4.Conclusions

We proved that selected microsatellite markers are eligible for authentication of food products of Oravaka tawny even for the F1 generations. This method could be the method of authentication and base line for Oravka products to be recognized as food with protected geographical indication.

The knowledge of genotyped parental generations let us predict and model the whole F1 production generation with all possible combinations of alleles from parental generation. The analysis of principal coordinates and neighbor joining (NJ) is showing, that modeled F1 generations are easily separated. In conclusion that means, we do not need to genotype a whole new generation. This created authentication method will be operational and functional for the F1 generation and products gained from those individuals. There is no need to genotype the F1 generation, because genetic distance is wide enough to reliably differ one population from another.

This bears great financial benefit for the authentication model. The need to observe and genotype only parental generation reduce the cost of microsatellite method. The method of microsatellite markes is proving to be fairly cheap and reliable for food authentication purposes.

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REDUCTION OF NITRITES ADDITION IN COOKED SAUSAGES FROM PHYTONUTRIENT SUPPLEMENTED PORK

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ABSTRACT

The aim of this study was to develop functional cooked sausages with reduced nitrites addition manufactured using pork after daily supplementation with 3.5 and 7.5 mg dihydroquercetin (DHQ) (samples D1 and D2, respectively) or 0.255 and 0.545 g dry distilled rose petals (*Rosa damascene* Mill.) (DDRP) (samples R1 and R2, respectively)/kg live weight/d. The sensory properties, colour characteristics (L*, a*, b*), TBARS and shelf life of the sausages were studied. Lower concentration of used phytonutrients approve sensory acceptance of sausages with half added nitrites. Higher doses of DHQ and DDRP supplements increased the sausage pH by 3% ($p < 0.05$). The use of 0.545 g DDRP /kg live weight/d as a feed supplement decreased the L* value and increased the redness (a*) in processed sausages while the higher doses of DHQ show an opposite effect. Feed enrichment with DHQ or DDRP is appropriate for manufacturing functional sausages with half added nitrites addition due to the stabilizing effect on colour (L*, a*, b*) characteristics in dynamics, the decreasing of TBARS and the increasing of the sausages shelf life.

1. Introduction

In recent years the development of novel functional foods has been focused on food industry. The increasing consumer health-care pays attention to the use of ingredients with proven anticancer, anti-mutagenic, antioxidant and delaying degenerative effect preventing the number of diseases in modern society (Kumar *et al.,* 2015). The first approach to creating functional meat products is the addition of natural supplements with a healthy effect such as vegetables, proteins, antioxidants, probiotics and prebiotics, soybeans, fruits, lactic acid bacteria etc. during manufacturing (Todra and Reig, 2011). Many consumers preferred meat products without allergens formulated by eliminating of the ingredients causing different types of allergies (Arihara, 2006).

Using the second approach, the composition of meat products can also be improved by animal feed enrichment. Feed supplement with conjugated linoleic acid impact on the accumulation of fatty acids and increase the nutritional value of the meat (Terpstra *et al.,* 2002). The feed enrichment with vitamin E inhibits the protein and lipid oxidation and improves the color stability (Cardinali *et al.,* 2015). Linseed and rapeseed oil increased the long-chain PUFAs in meat (Lopez-Ferrer *et al.,* 2001) and the addition of selenium is proven to increased its intramuscular content by 66% (Jiang and Xiong, 2016). Algae also have been used to

improve the nutritional value of feed (Christaki et al., 2010).

The use of herbs and spices like oregano (*Origanum vulgare L.*), rosemary (*Rosmarinus officinalis L.*) as well as sage (*Salvia officinalis L.*) shows a high antioxidant capacity (Cardinali *et al.,* 2015; Jiang and Xiong, 2016) and increases products shelf life.

Dihydroquercetin (DHQ) from Siberian larch (*Larix sibirica Ledeb*) is a natural antioxidant with proven antioxidant properties. DHQ inhibits the oxidation of LDL-cholesterol in blood serum (Artem'eva *et al.,* 2015).

The distilled rose petals are an interesting by-product, a waste material in rose oil and rose water production (*Rosa Damascena Mill.*). It is a typical Bulgarian raw material containing a wide range of antioxidant components - flavonoids with synergistic, antioxidative and antibacterial effect (Shikov *et al.,* 2012). According to Balev *et al.* (2015) the broiler feed supplemented with dry rose petals improves feed conversion.

In order to produce healthy meat products attempts have been made to reduce the nitrites during cooked sausages manufacturing (Vlahova-Vangelova *et al.*, 2014).

The production of meat with functional properties by a modeled chemical composition and an increase in the nutritional value after feed supplementing (Nieto *et al.,* 2010) is a challenge and innovation. The sausages manufactured with supplemented meat may have potential functional properties. Therefore, the purpose of this study was to develop a new strategy for processing of functional cooked sausages with half added nitrites manufactured by using pork obtained from pigs fed with supplements of 3.5 and 7.5 mg DHQ or 0.255 and 0.545 g DDRP/ kg live weight/ d.

2. Materials and methods

2.1. Feed supplements

The dihydroquercetin powder (96%) from Siberian larch (*Larix sibirica Ledeb*) was provided by Flavitlife Bio JSCo (Sofia, Bulgaria). Distilled rose petals were supplied by Bulattars Production Company Ltd (Sofia, Bulgaria), Pavel Banya, Stara Zagora district. The by-product after the rose oil distillation was pressed dried (60 °C, 6 h) and ground (< 0.4 mm).

2.2. Animal feeding, supplementation and harversting

The pigs (*Danube white breed*) were bred on the State Enterprise Experimental Farm at Agricultural Institute; Shumen, Bulgaria divided into five groups (one control and four experimental) each comprised of 8 animals. The animals received a typical commercial diet - libitum grower diet up to 60 kg live weight and a finisher up to 110 kg. After 155 day at an average live weight of 72 kg the pigs diets were supplemented for the last 40 days as follows: control (C) commercial diet without any supplement; sample D1 - commercial diet with 3.5 mg DHQ/kg live weight/d supplement; sample D2 - commercial diet with 7.5 mg DHQ/kg live weight/d supplement; sample R1 - commercial diet with 0.255 g DDRP/kg live weight/d supplement; sample R2 - commercial diet with or 0.545 g DDRP/kg live weight/d supplement.

After 40 days of supplemental feeding the pigs were transported and harvested at a processing plant (Unitemp Ltd., Voyvodinovo village, Plovdiv district, Bulgaria) in accordance with Council Regulations (EC) No 1/2005.

After 24 h chilling at 4°C each carcass was quartered at 12-13 rib, deboned and cold stored at 2 ± 1°C. The chilled (48 h) to 0 ± 4°C pork rump (pH 6.40) and pork chest (pH 6.5) were used for sausage production.

2.3. Sausage manufacturing

The sausages were produced in accordance with the requirements of the cooked meat product appropriate for EU (Table 1). Pork rump and pork chest obtained from five animal groups (C, D1, D2, R1, R2) were separately cut into pieces and used for the production of six sausage samples. Each filling mass was manufactured by mixing with salt and phosphates and blended in a cutter with an addition of flake ice. During sausages manufacturing the nitrites for experimental

groups D1, D2, R1, R2 were added in half. Pork rumps and pork chests from control group (C) were used for the production of two control samples: control C - with 100% nitrite addition and control sample C½ - with half-added nitrites. After filling in moisture and gases no-permeable five-layer polymer casings the sausages were cooked to an internal temperature of 72°C and chilled in cold water. The examinations were made dynamically on 1 and 7 day of the sausage refrigerated storage at 0 - ± 4°C.

The sodium chloride (salt), sugar and sodium nitrite (E250) were provided from the local market.

2.4. Methods

2.4.1. Sensory analysis

The sensory properties (cross sectional view, flavor, taste, color, texture) of the sausages were determined with a panel consisting of five members with proven tasting abilities (Meilgaard et al., 1999). The samples were scored using 1 to 5 scales.

2.4.2. Colour characteristics

The color properties CIE L*, a*, b* (Hunt et al., 2012) of the sausages on 1st day of storage at 0 ± 4°C were determined with Colorimeter Konica Minolta model CR-410 (Konica Minolta Holding, Inc., Ewing, USA), purchased by Sending, Inc. (Tokyo, Japan).

The changes of the color properties in the dynamics of the sausage surface cross-sectional views during the 60 min air exposure were examined on the 1st day of storage.

Table 1. Formulation of different samples functional cooked sausages

	Samples					
	C	**C½**	**D1**	**D2**	**R1**	**R2**
Feed supplementation	No suppl.	No suppl.	3.5 mg DHQ/ kg live weight/ d	7.5 mg DHQ/ kg live weight/ d	0.255 g DDRP/ kg live weight/ d	0.545 g DDRP/ kg live weight/ d
Sausage ingredients						
Pork rump, g/kg	500	500	500	500	500	500
Pork chest, g/kg	500	500	500	500	200	200
Flake ice, g/kg	200	200	200	200	200	200
Sodium chloride, g/kg	20	20	20	20	20	20
Polyphosphates, g/kg	2	2	2	2	2	2
Sodium nitrite, g/kg	0.10	0.05	0.05	0.05	0.05	0.05

2.4.3. pH value

The pH value of the samples was determined by pH-meter MS 2004, pH combination recorder S 450 CD (Sensorex pH Electrode Station, USA) (Young *et al.,* 2004).

2.4.4. 2-Thiobarbituric acid reactive substances (TBARS)

The double beam UV-VIS spectrophotometer M550 (Camspec Ltd, Cambridge, UK) was used for determination of the secondary products of the lipid oxidation expressed by malondialdehyde content (Botsoglou *et al*., 1994).

2.4.5. Microbiological assay

The samples for the microbiological assay were prepared by tenfold logarithmic dilution after homogenization with 90 mL of 0.85 % sodium chloride for 2 min at 200 min-1 (Merck

Bulgaria Joint-stock company, Sofia, Bulgaria) in stomacher bags (Seward Ltd, Worthing, West Sussex, UK). Once diluted, 1 cm^3 of the sample was added to sterile Petri plates (in triplicate for each dilution) with cooled to 45°C suitable agar (Sharma et al., 2005). The total viable count (TVC) was determined after 72h incubation at 28ºC on a plate count agar (PCA, Merck Bulgaria Joint-stock company, Sofia, Bulgaria) following the ISO 4833:2003 procedure and the count of yeast – after incubation of the same type on potato-dextrose agar Merck 1.10130 (Merck Bulgaria Joint-stock company, Sofia, Bulgaria) (Gelabert *et al.,* 2003).

2.4.6. Statistical analysis

Statistical analysis of the average values of five time reps was made. All statistical procedures for the data of different samples were analyzed by SAS software (SAS Institute, Inc. 1990). The Student-Newman-Keuls multiple range test was used to compare differences among means. The results were expressed as mean values and standard errors of the mean. A p-value less than 0.05 ($p < 0.05$) was considered as significant.

3.Results and discussions

3.1. pH value

pH in control sample C (Table 2), C½, as well as samples D1 does not differ significantly ($p > 0.05$). On the contrary, sausages from samples D2 and R2 had 3% higher pH ($p < 0.05$). For both used feed antioxidants the higher daily dose (7.5 mg DHQ/kg live weight/d or 0.545 g/kg DDRP/live weight/d increased the pH in sausages with half added nitrites. The results confirmed previous research that the feed supplements not only change the meat pH (Wiklund *et al.,* 2001) but also influence the pH of the manufactured sausages.

3.2. Sensory evaluation

The highest scores for cross sectional view and color were identified in samples C, C½ and the sausages from sample D1. The sensory panel confirmed the best flavor and taste in sausages from sample D1 followed by those from sample R1 and sample C½ (Fig. 1).

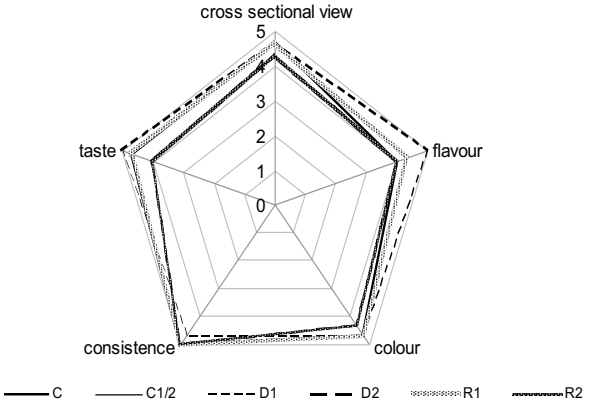

Figure 1. Sensory properties of studied sausages

The lowest flavor scores were obtained for sausages from samples D2 and R2. As many researchers exploring the effect of feed enriching on meat quality (Jerónimo *et al.,* 2009; Sobolev *et al.,* 2017) we can confirm that the concentration of the feed supplements is a very important factor for the sensory quality of pork functional sausages. It is clear that lower doses of DHQ in sample D1 and DDRP added in sample R1 had the positive effect on sensory acceptance of the produced sausages with half added nitrites (Fig. 1).

3.3. Colour characteristics (L*, a*, b*)

On the first day of storage (0 ± 4°C) the highest color lightness L* (Table 2) was established in sausages from sample D2 ($p < 0.05$). On the contrary, the lowest L* value ($p < 0.05$) was found in sausages from sample R2.

Closest to the control sample with 100% nitrites (C) was the colour lightness (L*) in sausages D1, while L* value in sample D2 was 2.3% higher ($p < 0.05$). Both the type and the concentration of supplements (DHQ or DDRP) durind pigs' feeding affect the colour lightness L* in produces sausages.

While higher doses of DDRP supplement reduced the L* value in sausages from sample R2, in D2 the L* value increased by 2.3% ($p < 0.05$) compared to C. Previous experiments with addition of rose petal extract in filling mass during sausages manufacturing, confirmed decrease in L* value of cut surface

compared to the 100% nitrite control sample, too (Vlahova-Vangelova et al., 2014).

Obviously, both the use of DDRP as feed supplement with subsequent sausage manufacturing and DDRP addition during sausage processing decreased the colour lightness L* in the produced sausages with half added nitrites.

Table 2. Changes in pH, colour (L*, a*, b*) characteristics, TBARS and microflora of studied sausage samples

		Samples					
	Day of storage	**C**	**C½**	**D1**	**D2**	**R1**	**R2**
pH	1	6,50 ^{a}±0,05	6,50 ^{a} ±0,05	6,50 ^{a} ±0,05	6,70 ^{b} ±0,05	6,60 ^{a,b} ±0,05	6,70 ^{b} ±0,05
L*	1	59,57 ^{a} ±0,04	58,97 ^{b} ±0,06	59,19 ^{c} ±0,03	61,12 ^{e} ±0,04	60,74 ^{d} ±0,12	58,43 ^{a} ±0,12
a*	1	11,76 ^{b} ±0,03	12,70^{d} ±0,59	12,39 ^{c} ±0,04	11,22 ^{a} ±0,01	11,25 ^{a} ±0,06	12,80 ^{d} ±0,02
b*	1	6,72 ^{d} ±0,06	5,32 ^{a} ±0,05	5,77 ^{b} ±0,04	6,98 ^{e} ±0,01	6,17 ^{c} ±0,02	6,18 ^{c} ±0,12
TBARS, mgMDA/kg	1	0,41^{a}±0,02	0,53 ^{b}±0,03	0,58^{b}±0,02	0,54^{b}±0,04	0,46 ^{a} ±0,03	0,42 ^{a} ±0,04
TBARS, mgMDA/kg	7	0,43 ^{a} ±0,04	0,75 ^{c}±0,07	0,67^{c} ±0,05	0,56 ^{b} ±0,04	0,51 ^{a,b} ±0,06	0,68^{c} ±0,09
TVC, log cfu/g	7	4,00 ^{a} ±0,20	4,70 ^{b}±0,10	4,30 ^{a,}±0,15	4,30 ^{a}±0,20	4,00^{a}±0,12	4,30 ^{a}±0,10
Yeasts and molds, log cfu/g	7	4,00 ^{a}±0,25	5,00^{b}±0,20	4,54^{a}±0,30	4,81^{a,b} ±0,15	4,54 ^{a}±0,30	4,70 ^{a,b}±0,10

$^{a, b, c}$ Means in the same row with different superscript letters differ significantly ($p < 0.05$).
SEM- standard error of the mean.

Similar effect on the colour lightness (L*) was reported by Salejda *et al*. (2017) exploring sausages manufactured with addition of powder sea buckthorn.

Compared to the sample C (Table 2) sausages with a higher dose of DHQ enriched meat (D2) as well as a lower supplement of DDRP (sample R1) had lower colour redness (a *) ($p < 0.05$).

On the contrary, in D1 and R2 sausages the a* value was found higher. Comparing the six studied samples (C, C½, D1, D2, R1,
R2) a* values in D2 and R1 samples were closest to controls C (with 100% nitrites addition) (Table 2). The conclusion was made that two used phytonutrients had different impact on the colour redness a*. The increase of DHQ concentration as supplement decreased the a* value, while the samples from meat enriched with higher concentration of DDRP (R2) significantly increased colour redness (a*) with 8.84% in pork cooked sausages. It seems that the type of used supplement as well as his concentration had the different impact on sausage colour characteristics. For example, Fernández-López *et al.* (2005) confirm the increasing of colour redness (a*) in beef meatballs manufacturing after addition of rosemary, garlic, orange, and lemon extracts. Contrary, Salejda *et al*. (2017) reported for reduction of colour lightness (L*) and redness (a*) after sausage processing with sea buckthorn. Even more, the authors (Salejda *et al*., 2017) found negative effect on sausage colour acceptance with the increasing of sea buckthorn concentration.

On the first day of storage (0 ± 4°C) the lowest b* value (of colour yellowness) was found on the cut surface of the sausages from sample C½ followed by samples D1, R1 and R2. Compared to control sample C (with 100% nitrites addition) the established decrease ($p < 0.05$) was 26%, 16.5%, 8.7% and 8.7%

respectively (Table 2). A decrease in the b * value after addition of rose petal extract during the sausages processing had been found previously by Vlahova-Vangelova *et al*. (2014), too. In sausages D2 and R1 processed with half added nitrites yellow color component b * was found to be closest to C.

3.4. TBARS

On the first day of the sausage storage (0 ± 4°C) the lowest TBARS (Table 2) was found in the controls C followed by sausages from DDRP enriched meat with half added nitrites (samples R1 and R2) ($p > 0.05$). Our results are in agreement with Karwowska and Dolatowski (2013) findings considered that the addition of mustard seed improve oxidation stability in pork sausages.

In comparison to C, after 7 days of storage (0 ± 4°C) sample C½ had two times higher content of TBARS ($p < 0.05$). The significant oxidative stability in lipid fraction was found in control C as well as in D2 and R1 sausages (Table 2). For the mentioned two samples TBARS does not change significantly ($p > 0.05$) for the seven-day storage period (0 ± 4°C).

Similar to our results were reported after oregano, rosemary, vitamin E (Cardinali *et al.,* 2015), oleic acid and tocopherol (Ventanas *et al.,* 2007) as well as natural phenolic antioxidants (Jiang and Xiong, 2016) feed supplementation. We can conclude that feed supplement with DHQ and DDRP rich in phenolic compounds decreases the formation of secondary products of lipid oxidation and can be used successfully for manufacturing of functional meat products with half added nitrites.

3.5. Microbiological analysis

The reduction of nitrites by 50% in sample C½ was not effective for the microbial growth suppressing (Table 2). The total viable count in sample C½ was 17.5% higher than in controls C (100% nitrite addition). No significant difference in the total viable count between controls C and sausages from other four samples D1 and D2 or R1 and R2. In comparison to sample C½ yeast and mold growth was suppressed in samples D1 and R1. Our data are in accordance with the results presented by Khan *et al.* (2011) about the beneficial effect of bioactive compounds and probiotics added as feed supplement or in filling mass on the shelf life of functional sausages. Many herbs and spices like black pepper, clove, oregano, thyme, (Dalle Zotte, 2016) cinnamon, onion, and garlic (Kreig, 2013) inhibit the microbial growth due to their essential oils content. The strong antimicrobial activity of rosemary was established due to the high level of phenolic antioxidants (Cardinali *et al.,* 2015). DHQ and DDRP as phytonutrients rich in phenolic compounds have strong antioxidant activity too (Shikov *et al.,* 2012; Artem'eva *et al.,* 2015).

The enrichment of the pigs' feed with dihydroquercetin (3.5 or 7.5 g/kg live weight/d) or dry distilled rose petals (0.252 or 0.545 g/kg live weight/d) inhibits the microbial growth in processed sausages during seven-day period of refrigerated storage (0 ± 4°C). The lower concentrations of phytonutrients as feed supplement (3.5 mg/kg DHQ or 0.252 g/kg DDRP live weight/d) had better impact for yeasts and moulds suppressing (Table 2).

3.6. Dynamics of the colour characteristics during 60 min exposure under air conditions

After 60 min of air exposure the most stable was color lightness L* in C and C½ followed by samples D2 and R1 (Table 3).

Table 3. Changes of colour lightness (L*), redness (a*) and yellowness (b*) in dynamics during the 60 minutes air exposure of sausages cut surface sectional views

L* value							
Time, min	**0**	**10**	**20**	**30**	**40**	**50**	**60**
C	59,57 ^{c,v}±0,04	59,28 ^{b,w} 0,05	59,31 ^{b,y} ±0,09	59,30^{b,y}±0,04	59,30 ^{b,x} ±0,03	59,28 ^{b,x} ±0,04	59,16 ^{a,x} ±0,03
C½	58,97 ^{c,w}±0,06	58,96 ^{c,y} ±0,01	58,96^{c,x}±0,01	58,94 ^{c,x} ±0,05	58,74 ^{b,v} ±0,05	58,72 ^{b,w} 0,03	58,52 ^{a,v} ±0,07
D1	59,19 ^{c,x} 0,03	59,34 ^{c,x} ±0,30	59,00 ^{b,x} ±0,07	58,94 ^{b,a,x} ±0,17	58,90 ^{b,w} 0,04	58,75 ^{a,w} ±0,05	58,78 ^{a,w} ±0,08
D2	61,12 ^{a,z}±0,04	61,62 ^{d,z} ±0,04	61,62 ^{d,z} ±0,04	60,77 ^{a,z} ±0,61	61,46 ^{c,z} ±0,04	61,45 ^{c,z} ±0,06	61,30 ^{b,y} ±0,03
R1	60,74 ^{a,y} 0,12	61,05 ^{b,z}±0,16	61,05 ^{b,z} ±0,06	61,28 ^{c,z} ±0,09	60,80^{a,y} ±0,03	60,73 ^{a,y} ±0,06	60,79 ^{a,z} ±0,08
R2	58,43 ^{b,c,v} 0,12	58,48 ^{d,v} ±0,01	58,48 ^{a,w}±0,02	58,46 ^{a,w} ±0,02	58,27 ^{b,u} ±0,08	58,37 ^{c,v} ±0,03	58,13 ^{a,u} ±0,02
a* value							
C	1,76 ^{g,x} ±0,03	10,91 ^{f,w} ±0,02	10,27 ^{e,x} ±0,02	9,76 ^{d,x} ±0,03	9,39 ^{c,x} ±0,02	9,08 ^{b,x}±0,02	8,86 ^{a,x} ±0,01
C½	2,70 ^{g,z} ±0,59	11,27 ^{f,x} ±0,01	10,60 ^{e,x} ±0,02	10,05 ^{d,x} ±0,03	7,93 ^{a,x} ±0,02	9,43 ^{c,x}±0,01	9,21 ^{b,x} ±0,01
D1	2,39 ^{g,y} ±0,04	11,55 ^{f,y} ±0,07	10,77 ^{e,x} ±0,03	10,19 ^{d,x} ±0,05	9,71 ^{c,x} ±0,01	9,39 ^{b,x} ±0,04	9,18 ^{a,x} ±0,02
D2	1,22 ^{f,w} ±0,01	10,41 ^{e,u} ±0,01	9,72 ^{d,x} ±0,02	9,90 ^{d,x} ±0,58	8,85 ^{c,x} ±0,02	8,58 ^{b,x} ±0,02	8,48 ^{a,x} ±0,01
R1	1,25 ^{g,w} ±0,06	10,50 ^{f,v} ±0,05	9,76 ^{e,x} ±0,01	9,22 ^{d,x} ±0,01	8,82 ^{c,x} ±0,01	8,58 ^{b,x} ±0,03	8,41 ^{a,x} ±0,01
R2	2,80 ^{g,z} ±0,02	11,87 ^{f,z} ±0,05	11,08 ^{e,x} ±0,05	10,48 ^{d,x} ±0,01	10,07 ^{c,x} ±0,02	9,78 ^{b,x} ±0,03	9,57 ^{a,x} ±0,01
b* value							
C	6,72 ^{a,y}±0,06	7,52 ^{b,y} ±0,02	7,73 ^{c,y} ±0,02	8,14 ^{d,z} ±0,02	8,30 ^{e,y}±0,01	8,49^{f,y}±0,03	8,66 ^{g,x} ±0,01
C½	5,32 ^{a,v}±0,05	7,18 ^{b,x} ±0,01	7,51 ^{c,v} ±0,01	7,81 ^{d,y} ±0,01	8,03 ^{e,v}±0,01	8,19 ^{f,v} ±0,01	8,34 ^{g,w}±0,02
D1	5,77 ^{a,w}±0,04	6,77 ^{b,v} ±0,01	7,15 ^{c,u} ±0,05	7,43 ^{d,x} ±0,02	7,65 ^{e,u} ±0,02	7,84 ^{f,u} ±0,01	7,96 ^{g,v} ±0,02
D2	6,98 ^{a,z}±0,01	7,64 ^{b,z} ±0,01	8,04 ^{c,z} ±0,01	8,03 ^{c,y,z} ±0,52	8,56 ^{c,z} ±0,02	8,79 ^{d,z} ±0,03	8,90 ^{d,z} ±0,02
R1	6,17 ^{a,x}±0,02	7,06 ^{b,w}±0,01	7,55 ^{c,w}±0,01	7,99 ^{d,y,z} ±0,29	8,07 ^{d,w}±0,02	8,24 ^{e,w} ±0,03	8,35 ^{f,w}±0,02
R2	6,18 ^{a,x}±0,12	7,09 ^{b,w}±0,02	7,61 ^{c,x} ±0,02	7,95 ^{d,y}±0,01	8,21 ^{e,x} ±0,03	8,42 ^{f,x} ±0,02	8,58 ^{g,y} ±0,01

$^{a, b, c}$ Means in the same row with different superscript letters differ significantly ($P < 0.05$).
SEM- standard error of the mean.

For the mentioned four samples (C, C½, D2, R1) the first L* value (0 min) and the last L* value (60 min) do not differ significantly ($p > 0.05$). The other two samples D1 and R2 show different trends. In sausages from sample R2 the color lightness L* significantly decreased by 33.7% ($p < 0.05$) for 60 min while in sample D1 L* value slightly increased.

The sausages from sample D1 are the only samples with color lightness increasing after a 60 minute of air exposure (Table 3).The cut surface redness a* traced after 60 min of air exposure decreased in all tested sausages but most significantly with 37.90% ($p < 0.05$) was the decrease in sample C½ (Table 2).

More stable was the a* value after 60 min of air exposure in samples D2, R1 and R2 with a decrease of 32.30%, 33.7% and 33.75% ($p < 0.05$) respectively. Similar changes were found in the colour yellowness b* studied in dynamics.

Once again the most significant decrease was established after 60 min of air exposure in the controls with half added nitrites - C½. The supplement with DHQ and DDRP in pigs' diet stabilized the a* and b* values of the manufactured sausages with half added nitrites. Our results show that after a feed enrichment with DHQ or DDRP the meat is suitable for processing the sausages with half nitrites addition and ensures stable colour (L*, a*, b*) characteristics (Table 3).

4. Conclusions

The use of pork supplemented with 3.5 mg or dihydroquercetin and 0.255 g dried distilled rose petals (*Rosa damascene Mill.*)/kg live weight/d improves sensory acceptance of produced sausages with half nitrites addition. The higher daily doses phytonutrients, namely 7.5 DHQ mg or 0.545 g DDRP/kg live weight/d increased the pH of the manufactured sausages. Our results show that after feed

enrichment with DHQ or DDRP the meat is suitable for sausages processing with half nitrites addition with stable colour (L*, a*, b*) characteristics. Pigs' feed enrichment with DHQ or DDRP decreased the formation of secondary products of lipid oxidation and increased the shelf life of the manufactured cooked sausages with half added nitrites and can be used successfully for development new functional cooked sausages.

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OLIVE LEAF EXTRACTS APPLICATION FOR SHELF LIFE EXTENSION OF VACUUM-PACKED FRIGATE MACKEREL (*AUXIS THAZARD*) FILLETS

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ABSTRACT

The current study was aimed at investigating the impact of olive leaf extracts (OLE) on the quality parameters of white and dark muscles from fillets of frigate mackerel (*Auxis thazard*) stored under vacuum pack (VP) at a refrigerated temperature (3±1°C). The sensory assessment indicated that both muscle types of frigate mackerel had 12 days of shelf-life, while the application of OLE extended the shelf-life by 3 and 6 days in the white and dark muscles, respectively. However, total volatile base-nitrogen exhibited a longer shelf-life in all samples, while thiobarbituric acid values showed shorter shelf-life for control samples. OLE application initiated the lipid peroxidation in white muscle at an early stage of storage and improved the microbiological quality of both muscles by reducing bacterial growth. An assessment of various texture related parameters indicated that VP alone could not enhance the textural quality in both muscles, while OLE improved adhesiveness and circumvented the softening of both frigate mackerel muscles.

1. Introduction

Fish has become an important source of human nutrition and is highly recommended by dieticians due to high-quality protein value and fatty acids profile. However, fish is a highly variable and perishable food due to the neutral pH, high water activity and simultaneous presence of microbial and enzymatic activities (Jeyasekaran *et al*., 2006). Traditionally, fish are preserved at low temperature to maintain quality. However, the inconvenience of dehydration, textural hardness, nutrient loss and reduced extractability of protein are some of the drawbacks associated with cold storage (Putro, 1989). During low-temperature storage, the lipid peroxidation takes place, and once it starts, it cannot be reverted and gets intensified when fish is filleted due to the increased surface area that comes into contact with oxygen. Therefore, it is necessary to develop efficient methods for preservation of seafood to avoid deterioration and maintain the fish quality in term of original color, texture, and flavor for a long period (Jeyasekaran *et al.*, 2006).

Vacuum packaging (VP) is one of the most widely used preserving methods that provide an oxygen-free environment. The preservative effect of VP is mainly limited to the prevention of lipid peroxidation and the growth of most spoiling microorganisms. However, even under such conditions, the growth of psychrotrophic

anaerobic or microaerophilic pathogens occurs, which is of great concern in terms of food safety and human health. The growth of the dominant bacteria associated with the fish spoilage, e.g., psychrotrophic lactic acid bacteria, results in unpleasant odors and a viscous appearance (Coma, 2008).

The addition of low levels of preservatives (natural or synthetic) with packaging is a desirable method of food preservation when combined with low temperatures; it could noticeably prolong the shelf-life of fish. Natural preservatives, obtained from plant products, are mainly used alone or in combination with other methods such as VP and low temperatures (Skandamis and Nychas, 2001) to enhance the sensory quality and extend the shelf-life of seafood and their derivative products. Such natural preservatives exert their effect through antimicrobial and antioxidant activities (Burt, 2004).

One of the most commonly used plants is olive (*Olea europaea* L.) and is widely cultivated throughout the Mediterranean region. Olive oil is the main product that is widely used in food, cosmetic, and therapeutic industries. In addition, the therapeutic properties of the olive plants are attributed to the antiseptic virtues of its leaves. Recently, scientific interest has grown in olive leaf extract (OLE) as a natural compound endowed with antimicrobial, antioxidant, anti-inflammatory, antihypertensive and other health benefits for human (Delgado-Adámez *et al.*, 2016). The natural antioxidant and antimicrobial properties of OLE may be explored to extend the shelf-life of fresh vacuum packed fish. There are several reports on the effect of the combination of natural preservatives and VP on the shelf life of different marine species (Kenar *et al.* 2010; Ucak *et al.* 2011; Houicher et al., 2013; Viji *et al.*, 2016; Chaves *et al.*, 2017; Lahreche *et al.*, 2019; El-Sohaimy *et al.*, 2019). However, to the best of our knowledge, no study has reported the differences between the combined effect of natural olive leaf extract and VP on the quality parameters of white and dark muscles of fish so far. Therefore, the present study was aimed to evaluate sensory, microbiological, chemical, and textural parameters of the white and dark muscle of frigate mackerel (*Auxis thazard*) fillets by applying OLE as a natural preservative followed by vacuum packaging and storage at a refrigerated temperature.

2. Materials and methods

2.1. Plants extract preparation

Fresh Olive leafs (*Olea europaea* L.) were dried in the dark at ambient temperature and finely ground. Extraction was accomplished in accordance with the method reported by Kenar *et al.* (2010) with slight modifications using absolute ethanol 96% (v/v). Ethanol from the filtrate was completely evaporated using a rotary evaporator (Heidolph Rotating Evaporator, VV2000, Schwabach, Germany), and the ethanol-free filtrate was kept in the dark at −18°C till usage. Before using, the extract was treated by UV-light (30W, 50cm irradiation distance) using a laminar flow cabinet (Telstar Bio-II-A, Telstar, Madrid, Spain) for 30 min to eliminate any existing microflora.

2.2. Fish sample preparation

Frigate mackerel (*Auxis thazard*) was caught from Mediterranean Sea; Turkey and was stored in ice for approximately 6 hours post-capture. On arrival at the laboratory, fish were immediately gutted, beheaded, filleted without skin separation and rinsed with tap water. The average weight, length, width and thick of the obtained fish fillets were 151.53±26.88g, 20.31±1.15cm, 8.01±0.51cm and 1.46±0.21cm, respectively (*n=10*). After that, fillets were randomly allocated into two lots. One lot kept as control (untreated) and the second lot was dipped in a sterile distilled water containing 0.5% of sterilized OLE for 5 min (treated). All lots were vacuum packaged in bags of polyamide film (Polinas, Manisa, Turkey) with 90 µm of film thickness, 8.5 $g/m^2/24h$ of water permeability and 160 $cm^3/m^2/24h$ oxygen permeability. All groups were stored as intact fillet at 3±1°C. Sensory

characteristics, chemical changes, microbial spoilage and texture analysis were assessed, in triplicate, on days 0, 4, 8, 12, 15, and 18 in separated white and dark muscles obtained from the same fillet.

2.3. Chemical analysis and pH

pH values were recorded in accordance with Woyewoda *et al.* (1986) method. Total volatile basic nitrogen (TVB-N) content was accomplished by using the Antonocopoulos (1973) method. Peroxide value (PV) was done by AOCS (1994) method. Thiobarbituric acid reactive substances value (TBARs) was achieved in line with Tarladgis *et al.* (1960) method. Free fatty acid analysis (FFA) was carried out by the AOCS (1994) procedure.

2.4. Microbiological analysis

Preparation of test samples from fish muscles, initial suspension and serial decimal dilutions for microbiological analysis were prepared using sterile quarter-strength Ringer's solution (Fluka; Sigma Aldrich, Sweden) (ISO., 2017). Aliquot of 0.1 ml from each dilution was spread gently onto the surface of the dry media (ISO., 2013; ISO., 2001). The enumeration of aerobic mesophilic and psychrophilic bacteria was performed in plate count agar (Fluka 70152; Steinheim, Switzerland) after incubation for 72h at 30°C (ISO., 2013) and for 10 days at 6.5°C (ISO., 2001), respectively. All analysis was performed in triplicate and was taken from each of dark and white muscles of each of two different groups. All microbiological data are expressed as a logarithm of colony forming units per gram of sample.

2.5. Sensory analysis

Quality Index Method (QIM) established by Ariyawansa *et al.* (2003) for filleted fish was used with some modifications for sensory assessment. The scheme contained five quality parameters (e.g. surface and skin appearance, color, texture and odor of fillet, and plant extract odor). Generally, the scheme had a score system for each of parameters of 4 demerit points ranged from 0 (best quality) to a maximum of 3 (poorer quality). The total sum of demerit point was 15. The Panel, consisting of six trained evaluators, inspected fillets and recorded the appropriate demerit point for each of parameters and then the scores of all parameters are summed to give an overall demerit score, so-called quality index score. The panel was requested to state whether the fish were acceptable or not for the determination of the shelf-life of frigate mackerel fillets.

Sensory evaluations of cooked frigate mackerel were assessed following to the same method of Ariyawansa *et al.* (2003) without modification. Fish fillets were cooked in a microwave for 4 min (700W) and then served hot. Panelists scored for odor and flavor, using a torry score with eight-point hedonic scale (3, poorest quality to 10, best quality). The rejection threshold set at ≤ 6.

2.6. Texture Profile Analysis (TPA)

Texture profile analysis (TPA) was done in line with the modified procedure of Liu *et al.* (2014) in triplicate using a texture analyzer TA-XT Plus equipped with a load cell of 50 N and the software was Texture Expert, v1.20 (Stable Micro Systems Ltd, Godalming, Surrey, UK). Before analysis, samples having a length, height and thickness: 35mm, 20mm and 10mm, respectively were prepared from each of white and dark muscles. A flat-ended cylinder (P/36R) with a diameter of 35mm was used at a constant speed of 1 mm/s until it accomplished 50% of the sample thickness. After that, the force was decreased and the sample was able to rebound for 10 seconds leaving the cylinder just touching the surface, then the cylinder was pressed on the sample a second time. From the resulting force/deformation graphs, five textural parameters including hardness, springiness, adhesiveness, resilience and chewiness were estimated.

2.7. Statistical analysis

All mean values and standard deviations were determined from triplicate data obtained

for each of muscle type from each lot. Analysis of variance (ANOVA) was applied using the Statistical Package for Social Science (SPSS 19.0 for windows, SPSS Inc., Chicago, IL, USA) followed by Duncan's Multiple Range Tests at P value of <0.05 were carried out to conclude the significant differences.

3.Results and discussions

3.1. Chemical analysis and pH

Table 1 summarizes the variations in pH and chemical parameters on each sampling day. The initial pH of dark muscles was significantly higher ($p<0.05$) than that of white muscles. Similar values were also found by Junsi *et al.* (2012) for dark muscle of skipjack tuna (*Katsuwonus pelamis*). On the contrary, Chaijan *et al.* (2013) reported that the initial pH of the same species was higher in white muscles than the dark ones, indicating that pH may vary not only among fish species but also within the same species, which might be due to the several factors including season, harvesting procedures, biological condition, and methods of killing (Mazorra-Manzano *et al.*, 2000). Throughout the experiment, pH values of dark muscles were significantly higher ($p<0.05$) than those of white muscles, irrespective of treatment. This may happen due to the breakdown of glycogen to lactic acid that must have occurred in white muscle due to the intense activity/stress during capture resulting in lowered pH (Modigh and Tota, 1975). In all experiment, changes in pH showed the same tendency in both muscles during the storage. In the treated samples, pH reached to the maximum level in both muscles only on the 4th day of storage. The increase in pH may be caused by the accumulation of alkaline compounds such as ammonia and trimethylamine (Ruiz-capillas and Moral, 2005) and/or biogenic amines mainly produced by fish spoiling bacteria (Krízek *et al.*, 2004). The decrease in pH may also be attributed to the anaerobic glycogen decomposition occurring in fish (Song *et al.*, 2011). No differences ($p>0.05$) between the treatments were observed in dark muscles. At the end of storage, pH in all groups declined significantly ($p<0.05$) in both muscle types, probably due to the growth of lactic acid bacteria (Li *et al.*, 2016). These results inferred that the application of OLE along with VP could affect the pH value of frigate mackerel muscles; however, the pH value should not be considered as an important index to determine the freshness of the fish.

The TVB-N values were increased with storage time and generated by the microbial spoilage and enzymatic activity; however, this rise does not reveal the mode of spoilage and depend greatly on the analytical method (Song *et al.*, 2011; Mazorra-Manzano *et al.*, 2000). Initially, TVB-N values were significantly lower ($p<0.05$) in white muscles than dark muscles and are in line with the findings by Liu *et al.* (2014). This might be due to the fact that dark muscle is generally rich in vitamin and minerals, and hence a good source of nutrients for microbial growth (Wendakoon *et al.*, 1990). In our study, TVB-N values increased with time in both muscle types for all packaged samples followed by a decline, which indicates a reduction in bacterial activity at the end of storage. The TVB-N data marked significant differences ($p<0.05$) between both muscle types throughout the storage time for control samples and until 12 days of storage for the treated samples. Over the storage period, TVB-N values of control samples were significantly lower ($p<0.05$) in white muscle than dark muscle, indicating that white muscle spoiled slowly than dark muscle. However, the application of OLE showed a protective effect on fish fillets and yielded the lowest ($p<0.05$) TVB-N values in both muscles than the control. The noticeable reduction in TVB-N in the treated samples may be associated with the effect of the combination of VP with the antibacterial properties of the phenolic compounds present in OLE such us oleuropein, verbascoside, and hydroxytyrosol (Benavente-García *et al.*, 2000). Throughout the investigation, only the control sample exceeded the acceptability limit (30 mg TVB-N/100g: Liu *et al.*, 2014) on the 15 days reaching the TVB-N of 30.65 and 30.11 mg/100g for the

white and dark muscles, respectively. In contrast, TVB-N values of both muscles in the treated samples were below this limit until the end of the storage and carried lower values than the control samples. Thus, TVB-N may potentially be a suitable index for assessing the spoilage of filleted frigate mackerel stored at a refrigerated temperature under VP conditions.

Table 1. Effect of OLE on pH, TVB-N (mg/100g of sample), PV (mEq/kg of fat), TBA (mg malonaldehyde/kg of the sample) and FFA (oleic acid %) in white and dark muscles of vacuum-packed frigate mackerel stored at a refrigerated temperature (3±1°C).

Storage time (days)		**0**		**4**		**8**	
Parameters	**Samples**	**W**	**D**	**W**	**D**	**W**	**D**
pH	**Control**	5.69 ± 0.01^{Y}	5.77 ± 0.02^{X}	$5.73\pm0.04^{a,Y}$	$5.89\pm0.06^{a,X}$	$5.85\pm0.04^{a,X}$	$5.89\pm0.03^{a,X}$
	OLE			$5.82\pm0.09^{a,Y}$	$5.95\pm0.07^{a,X}$	$5.77\pm0.05^{b,Y}$	$5.89\pm0.03^{a,X}$
TVB-N	**Control**	12.82 ± 0.41^{Y}	17.21 ± 0.84^{X}	$18.94\pm0.70^{a,Y}$	$28.29\pm0.88^{a,X}$	$21.41\pm0.73^{a,Y}$	$22.96\pm0.63^{a,X}$
	OLE			$17.24\pm0.63^{a,Y}$	$20.36\pm1.72^{b,X}$	$15.70\pm0.70^{b,Y}$	$17.39\pm0.77^{b,X}$
PV	**Control**	2.61 ± 0.23^{X}	1.45 ± 0.20^{Y}	$3.83\pm0.04^{b,X}$	$1.88\pm0.07^{a,Y}$	$6.33\pm1.15^{a,X}$	$0.99\pm0.02^{b,Y}$
	OLE			$8.12\pm1.86^{a,X}$	$1.70\pm0.08^{a,Y}$	$2.34\pm0.49^{b,X}$	$1.22\pm0.10^{a,Y}$
TBA	**Control**	0.56 ± 0.01^{Y}	0.64 ± 0.01^{X}	$0.84\pm0.04^{a,X}$	$0.57\pm0.02^{a,Y}$	$0.91\pm0.06^{a,X}$	$0.66\pm0.01^{b,Y}$
	OLE			$0.68\pm0.02^{b,X}$	$0.62\pm0.06^{a,X}$	$0.75\pm0.08^{b,Y}$	$0.90\pm0.02^{a,X}$
FFA	**Control**	3.73 ± 0.05^{Y}	11.54 ± 0.15^{X}	$6.55\pm0.30^{a,Y}$	$9.47\pm0.83^{a,X}$	$7.80\pm0.17^{a,Y}$	$18.83\pm0.90^{a,X}$
	OLE			$6.40\pm0.46^{a,Y}$	$7.99\pm0.53^{b,X}$	$6.11\pm0.05^{b,Y}$	$17.14\pm0.19^{b,X}$

Values are expressed as means ±SD, (n =3), W: white muscle, D: dark muscle.
a,b Different lowercase letters in the same column indicate significant differences ($P < 0.05$) between samples in the same muscle.
X,Y Different capital letters in the same row indicate significant difference ($P < 0.05$) between two muscles in the same sample.

Table 1. Effect of OLE on pH, TVB-N (mg/100g of sample), PV (mEq/kg of fat), TBA (mg malonaldehyde/kg of the sample) and FFA (oleic acid %) in white and dark muscles of vacuum-packed frigate mackerel stored at a refrigerated temperature (3±1°C). (continued).

Storage time (days)		**12**		**15**		**18**	
Parameters	**Samples**	**W**	**D**	**W**	**D**	**W**	**D**
pH	**Control**	$5.87\pm0.02^{a,Y}$	$5.93\pm0.02^{a,X}$	$5.88\pm0.02^{a,X}$	$5.92\pm0.02^{a,X}$	$5.76\pm0.02^{b,Y}$	$5.82\pm0.02^{a,X}$
	OLE	$5.73\pm0.07^{b,Y}$	$5.88\pm0.05^{a,X}$	$5.83\pm0.01^{b,Y}$	$5.89\pm0.03^{b,X}$	$5.80\pm0.03^{a,X}$	$5.81\pm0.02^{a,X}$
TVB-N	**Control**	$19.59\pm0.04^{a,X}$	$19.25\pm0.20^{a,Y}$	$30.65\pm0.07^{a,X}$	$30.11\pm0.28^{a,Y}$	$26.80\pm0.71^{a,Y}$	$29.31\pm0.52^{a,X}$
	OLE	$18.43\pm0.63^{b,X}$	$17.97\pm0.67^{b,X}$	$28.03\pm0.65^{b,X}$	$27.38\pm1.14^{b,X}$	$20.76\pm0.77^{b,X}$	$20.71\pm0.90^{b,X}$
PV	**Control**	$3.23\pm0.26^{a,X}$	$1.62\pm0.01^{a,Y}$	$4.37\pm0.99^{a,X}$	$5.32\pm0.25^{a,X}$	$5.27\pm0.65^{a,X}$	$2.16\pm0.04^{a,Y}$
	OLE	$1.77\pm0.31^{b,X}$	$1.35\pm0.05^{b,Y}$	$4.18\pm0.50^{a,X}$	$1.90\pm0.15^{b,Y}$	$1.37\pm0.07^{b,Y}$	$2.41\pm0.25^{a,X}$
TBA	**Control**	$1.46\pm0.01^{a,X}$	$1.12\pm0.01^{a,Y}$	$0.89\pm0.01^{a,X}$	$0.63\pm0.02^{a,Y}$	$1.28\pm0.02^{a,X}$	$1.31\pm0.01^{a,X}$
	OLE	$1.34\pm0.02^{b,X}$	$0.99\pm0.01^{b,Y}$	$0.67\pm0.01^{b,X}$	$0.60\pm0.01^{b,Y}$	$1.17\pm0.01^{b,X}$	$0.95\pm0.02^{b,Y}$
FFA	**Control**	$8.28\pm0.24^{a,Y}$	$15.65\pm0.83^{a,X}$	$8.42\pm0.03^{a,Y}$	$18.25\pm0.06^{a,X}$	$10.30\pm0.04^{a,Y}$	$20.53\pm0.37^{a,X}$
	OLE	$7.16\pm0.18^{b,Y}$	$14.06\pm0.36^{b,X}$	$8.27\pm0.14^{a,Y}$	$14.32\pm0.09^{b,X}$	$7.78\pm0.20^{b,Y}$	$15.99\pm0.13^{b,X}$

Values are expressed as means ±SD, (n =3), W: white muscle, D: dark muscle.
a,b Different lowercase letters in the same column indicate significant differences ($P < 0.05$) between samples in the same muscle.
X,Y Different capital letters in the same row indicate significant difference ($P < 0.05$) between two muscles in the same sample.

Peroxide value (PV) characterizes the level of primary lipid peroxidation products. As indicated in Table 1, PV was significantly higher ($p<0.05$) in white muscle than dark muscle during all storage period. These results did not concord with those reported by Sohn

and Ohshima (2010) and this may probably be due to the use of intact fillet with skin. PV did not exceed the pre-established limit of 20 mEq O2/kg of fat in all packaged samples. This may be attributed to the use of VP that involved complete removal of oxygen with hermetic sealing and led to a significant reduction in lipid peroxidation during the storage (Johnson and Decker, 2015). At the earlier stage, the combination of plant extracts and VP reduced peroxide formation. Throughout the experiment, PV was lower ($p<0.05$) in the treated groups than control, and remarkably less in the dark muscles suggesting that the combined application of plant extract and VP effectively delayed the lipid peroxidation in both muscles. However, on day 4, PV of only white muscles rose substantially in the treated sample and was significantly higher ($p<0.05$) than the control exhibiting a pro-oxidant effect, rather than an antioxidant. This may be ascribed either to the use of lower concentrations of the extract or to the catalytic effect of chlorophyll and their derivatives that promote lipid peroxidation during storage (Jukic *et al.*, 2015). Alternatively, no differences ($p>0.05$) were observed in PV among the dark muscle for all samples until 8 days of the storage. The observed stability in dark muscle may be related to the use of intact fillet. From these observations, it can be concluded that the major effect on reducing peroxide values is attributed to the use of VP. The application of OLE seemed to initiate lipid peroxidation only in white muscle at the early stage of storage.

TBA is a measure of malonaldehyde (MDA) content, which is a secondary end product of lipid hydroperoxide breakdown. In the present study, higher initial TBA values were observed in dark muscle than white muscle (Table 1), which might be due to the higher lipid content in dark muscles. However, Liu *et al.* (2014) reported a lower TBA values in white and dark muscles of skipjack tuna. In our study, MDA-content white muscles were significantly higher ($p<0.05$) than dark muscles after 4 days of storage suggesting that the rate of hydroperoxide decomposition was relatively faster in white muscle throughout the storage period. This could be attributed to the refrigerated condition, to the removal of oxygen and to the use of intact fillet. In the present study, TBA values of all the samples showed a trend to an increase in both muscle types during the storage, followed by a subsequent decline, which was not stable for the later stage of storage. The increase in MDA-content indicated the formation of secondary lipid oxidation products, whereas the decrease in the values may be indicative of an interaction between MDA and proteins, amino acids, glycogen, etc. (Kolakowska, 2002). Irrespective of the sampling day, for both the muscle types, a higher TBA value ($p<0.05$) was recorded in controls as compared to the treated samples. On the other hand, a reverse of this was observed between samples on day 8 only for dark muscle and apparently led to TBA formation at the earlier stage of storage. A TBA value of 1–2 mg MDA/kg of fish flesh is considered as the threshold limit beyond which fish normally develop an unpleasant flavor (Connell, 1990). In both treated muscles, TBA-values exceeded 1 mg MDA/kg at day 12 of storage; however, MDA-content in both muscles from these samples did not comply with the sensory assessment. Throughout the storage period and especially on day 12, panelists did not detect any foul odor or taste in the treated samples, probably since TBA values did not exceed the minimum detectable level by tasters, which is estimated at 1.44 mg MDA/kg (Ruiz-Capillas and Moral, 2001).

The main objective of using natural extract is to delay the onset of lipid oxidation and oxidative product accumulation. However, it is essential to understand that the muscle type, oxygen availability, and plant extract application, as well as other factors that can affect fat content in fish, may also have an impact on the variation in TBA-content in fishery products and therefore must be taken into consideration during storage. On the basis of TBA data, it can be concluded that the application of OLE (0.5%) exhibited an

antioxidant effect on frigate mackerel muscles. However, further investigations must be carried out to elucidate the mechanism by which the OLE derivatives delay the lipid oxidation.

FFA is an index of hydrolytic activity. Throughout the experiment, regardless of the treatments, there were recorded significant differences ($p<0.05$) in FFA values between white and dark muscles (Table 1). Lipid hydrolysis was relatively faster in dark muscles than the white muscles. This might be attributed to the higher lipid content and lipase activity present in dark muscle (George and Bokdawala, 1964). In this study, FFA increased with storage time, and the loss of freshness related to hydrolytic activity might occur because of the lipase and phospholipase activity (Quitral et al., 2009). However, a noticeable decline, followed by an increase, was observed only in dark muscle at day 4 and 12, indicating that the rate of loss succeeded in the rate of FFA production. The loss of FFA during storage is probably due to the increased lipid oxidation, to the interaction with proteins and/or it could be related to the growth of some microorganisms that can use the FFA as an energy source (Anderson and Ravesi, 1968). On the other hand, during the storage, lipid hydrolysis in both muscle types was found higher ($p<0.05$) in control than the treated samples except for white muscles. No differences ($p>0.05$) in FFA concentrations were found between treatments at 4 and 15 days. This may happen due to the phenolic compounds present in the plant extracts, which inhibit the activity of FFA liberating enzymes. Hence, the combination of OLE with VP effectively delayed the FFA formation in both muscle types.

3.2. Microbiological assessments

The changes in microbial growth of the white and dark muscles of frigate mackerel fillets treated with OLE under vacuum packaging at a refrigerated temperature are presented in Table 2.

Table 2. Effect of OLE on the Mesophilic and Psychrophilic bacterial counts in white and dark muscles of vacuum-packed frigate mackerel stored at a refrigerated temperature (3±1°C).

Storage time (days)		**0**		**4**		**8**	
Bacteria	**Samples**	**W**	**D**	**W**	**D**	**W**	**D**
Mesophilic aerobic bacteria (log cfu/g)	**Control**	2.59 ± 0.16^{Y}	3.31 ± 0.15^{X}	$3.55\pm0.08^{a,X}$	$3.82\pm0.20^{a,X}$	$3.89\pm0.36^{a,X}$	$4.01\pm0.06^{a,X}$
	OLE			$3.51\pm0.25^{a,X}$	$3.24\pm0.09^{b,X}$	$3.81\pm0.13^{a,X}$	$3.94\pm0.44^{a,X}$
Psychrotrophic bacteria (log cfu/g)	**Control**	2.95 ± 0.06^{X}	2.86 ± 0.10^{X}	$3.74\pm0.31^{a,X}$	$3.56\pm0.32^{a,X}$	$4.12\pm0.12^{a,X}$	$3.89\pm0.16^{a,X}$
	OLE			$3.32\pm0.23^{b,X}$	$3.07\pm0.56^{a,X}$	$3.46\pm0.15^{b,X}$	$3.45\pm0.09^{b,X}$

Values are expressed as means ±SD, (n =3), W: white muscle, D: dark muscle.
a,b Different lowercase letters in the same column indicate significant differences ($P < 0.05$) between samples in the same muscle.
X,Y Different capital letters in the same row indicate significant difference ($P < 0.05$) between two muscles in the same sample

Table 2. Effect of OLE on the Mesophilic and Psychrophilic bacterial counts in white and dark muscles of vacuum-packed frigate mackerel stored at a refrigerated temperature (3±1°C) (continued).

Storage time (days)		**12**		**15**		**18**	
Bacteria	**Samples**	**W**	**D**	**W**	**D**	**W**	**D**
Mesophilic aerobic bacteria (log cfu/g)	**Control**	$5.07\pm0.23^{a,X}$	$4.93\pm0.13^{a,X}$	$7.05\pm0.04^{a,X}$	$6.74\pm0.38^{a,X}$	$7.39\pm0.17^{a,X}$	$7.62\pm0.14^{a,X}$
	OLE	$4.63\pm0.27^{a,X}$	$4.59\pm0.41^{a,X}$	$5.48\pm0.22^{b,X}$	$4.87\pm0.\ 30^{b,Y}$	$5.87\pm0.31^{b,X}$	$5.84\pm0.26^{b,X}$
Psychrotrophic bacteria (log cfu/g)	**Control**	$4.85\pm0.42^{a,X}$	$4.64\pm0.30^{a,X}$	$5.75\pm0.21^{a,X}$	$6.04\pm0.08^{a,X}$	$5.93\pm0.10^{a,X}$	$6.01\pm0.23^{a,X}$
	OLE	$4.81\pm0.20^{a,X}$	$4.55\pm0.43^{a,X}$	$5.40\pm0.35^{a,X}$	$5.15\pm0.21^{b,X}$	$5.82\pm0.11^{a,X}$	$6.07\pm0.18^{a,X}$

Values are expressed as means ±SD, (n =3), W: white muscle, D: dark muscle.
a,b Different lowercase letters in the same column indicate significant differences ($P < 0.05$) between samples in the same muscle.
X,Y Different capital letters in the same row indicate significant difference ($P < 0.05$) between two muscles in the same sample

The initial low microbial loads observed in this study indicated the good quality of the fish. During the storage, microbial load increased significantly ($p<0.05$) with time. Statistical analysis showed that both mesophilic and psychrotrophic bacterial loads were not affected by the muscle type in all treatments, even in the control, which may be due to the elimination of oxygen from the package. The effect of OLE on the mesophilic bacterial load could only be determined in both muscles at the later stages (15 and 18 days). However, the growth of psychrotrophic bacteria was faster in the controls than the treated samples only at the early stage of storage (day 4 and 8). In the control samples, regardless of the muscle types, both mesophilic and psychrotrophic bacteria loads exceeded 6 $\log_{10}$ CFU/g at day 15 of storage. On the other hand, both mesophilic and psychrotrophic bacterial loads reached this limit at day 18 in both muscles in the treated groups conferring an extension of 3 days in microbiological shelf-life for samples treated with OLE. This advocates that the treatment with OLE improved the microbiological quality of both muscle types but it was less effective in reducing mesophilic and psychrotrophic bacterial growth, probably due to the low concentration of the extract.

3.3. Sensory assessments

Sensory assessment of raw and cooked white and dark muscles of frigate mackerel fillets stored under vacuum-packaging with or without OLE was evaluated throughout the refrigerated storage period. The total demerit points of the white and dark muscle of frigate mackerel fillets are presented in Fig. 1. Initially, sensory score of all batches were zero, indicating the absolute freshness. The demerit points of both muscles increased with storage time and the quality of fish declined. The loss of freshness during storage was recorded higher in both muscles from control sample compared to their treated counterparts. No significant differences ($p>0.05$) were observed between the white and dark muscles irrespective of the treatment. Considering the sensory score of 8 as the limit of acceptability, the observed shelf-life of both muscles of the control group was no longer than 12 days.

Combination with VP enhanced the organoleptic quality of fish. It improved the quality parameters of fillets by 3 more days in white muscle, whereas an extension of 6 days was observed in the dark muscle compared with their control counterparts (total demerit points were 8.11 and 8.36, respectively). Therefore, OLE had a major enhancement in sensory quality of dark muscle and extended the shelf-life of frigate mackerel. Thiansilakul *et al.* (2011) reported that the use of vacuum-packaging for the storage of Eastern little tuna fillet extended the acceptable appearance for 3 days more than those maintained in air pack condition. Thus, the observed extension in shelf-life of both muscles of frigate mackerel may a result of the cumulative effect of OLE application at 0.5% (w/v) and VP during the storage at a refrigerated temperature.A sensory score of the cooked frigate mackerel was also assessed separately in both muscles throughout the 18 days of storage (Fig. 2). On each sampling day, significant differences ($p<0.05$) were observed between the control and treated batch in each muscle type; however, the differences observed between both muscles for each batch were no significant ($p>0.05$). Based on the limit of acceptability of >6, the sensory quality of both muscle types declined in intensity during storage and exceeded this limit at 12 days for control (demerit scores; 5.82 and 5.69, respectively) and 15 days for treated samples sample (demerit scores; 6.13 and 5.77, respectively). At the rejection time, general acceptability scores of the cooked white muscle of frigate mackerel fillets were in agreement with their corresponding raw samples, while dark muscle showed an additional extension of 3 days compared to their homologous cooked samples. This might occur due to the undesirable flavor acquired by OLE after cooking. These findings indicate that the combination of OLE and VP may prolong the shelf-life of both muscle types of frigate mackerel.

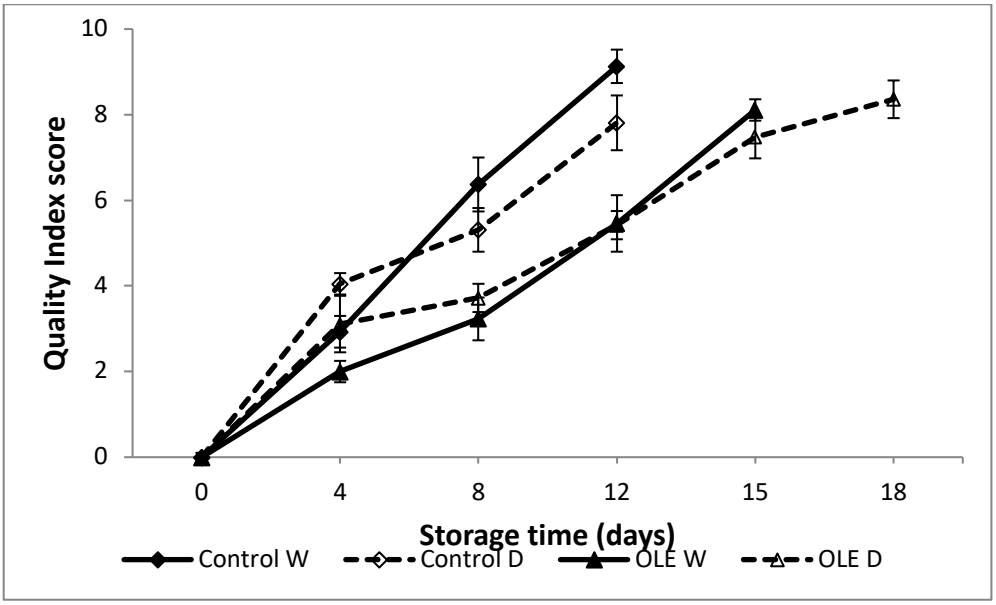

Figure 1. Sensory assessment of the raw white (W) and dark (D) muscles from vacuum-packed frigate mackerel fillets during the storage at a refrigerated temperature: Untreated (Control), Olive leaf extract (OLE). *Mean scores of sensory assessment (n=6). Standard deviations are indicated by bars*.

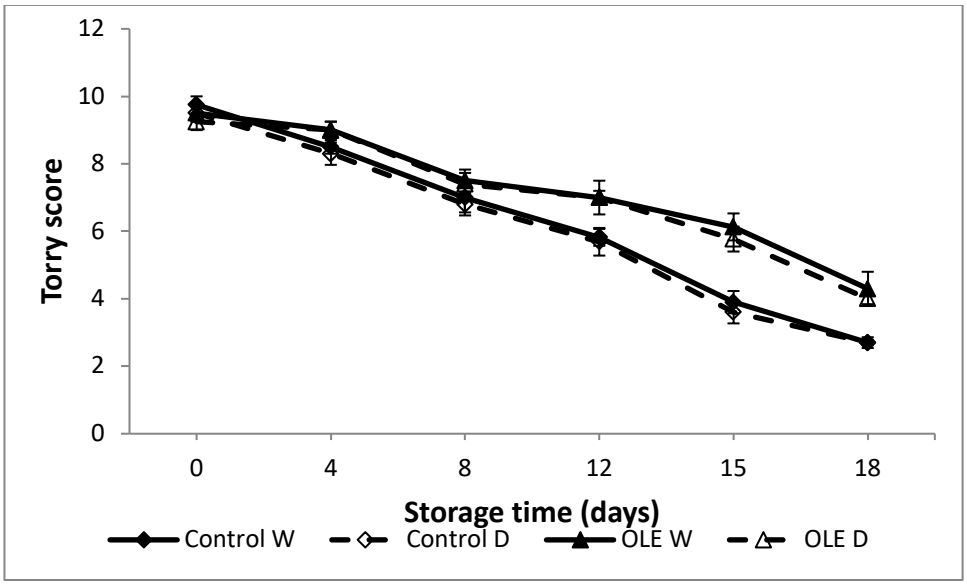

Figure 2. The sensory score of cooked white (W) and dark (D) muscles from vacuum-packed frigate mackerel fillets during the storage at a refrigerated temperature: Untreated (Control), Olive leaf extract (OLE). *Mean scores of sensory assessment (n=6). Standard deviations are indicated by bars.*

3.4. Texture Profile Analysis

TPA results of frigate mackerel fillets during storage are given in Table.3. The hardness (firmness) reduced significantly ($p<0.05$) with storage time, indicating that VP alone cannot preserve the hardness of fillet. Initially, both muscles had the same firmness; however, during the experiment, hardness of white muscles became significantly higher ($p<0.05$) than that of dark muscles. This could be illustrated by the differences in proximate

composition, enzymatic activities, proteolytic activities and/or the changes in post-mortem pH, which extremely affect the properties of the connective tissue and determine the texture of the muscle (Jinadasa *et al.*, 2015). Both muscles of the treated groups were significantly harder ($p<0.05$) than their control counterparts throughout the storage, revealing that the combined use of OLE with VP can prevent the softening of fish muscles.

Table 3. Effect of OLE on texture profile analysis in white and dark muscles of vacuum-packed frigate mackerel stored at a refrigerated temperature (3±1°C)

Storage time (days)		**0**		**4**		**8**	
Parameters	**Samples**	**W**	**D**	**W**	**D**	**W**	**D**
Hardness (N)	**Control**	45.17 ± 0.05^{X}	45.02 ± 0.11^{X}	$44.49\pm0.37^{b,X}$	$43.11\pm0.10^{b,Y}$	$40.08\pm0.81^{b,X}$	$38.14\pm0.08^{b,Y}$
	OLE			$45.26\pm0.01^{a,X}$	$44.16\pm0.04^{a,Y}$	$43.29\pm0.24^{a,X}$	$40.23\pm0.16^{a,Y}$
Springiness (Ratio)	**Control**	1.90 ± 0.03^{Y}	1.97 ± 0.02^{X}	$1.59\pm0.03^{b,Y}$	$1.98\pm0.01^{a,X}$	$1.70\pm0.02^{a,X}$	$1.70\pm0.02^{a,X}$
	OLE			$1.78\pm0.04^{a,X}$	$1.67\pm0.03^{b,Y}$	$1.77\pm0.05^{a,X}$	$1.73\pm0.01^{a,X}$
Adhesiveness (mJ)	**Control**	0.21 ± 0.03^{Y}	0.43 ± 0.02^{X}	$0.12\pm0.03^{a,Y}$	$0.31\pm0.03^{b,X}$	$0.11\pm0.04^{b,Y}$	$1.19\pm0.02^{a,X}$
	OLE			$0.18\pm0.03^{a,Y}$	$1.25\pm0.02^{a,X}$	$1.14\pm0.05^{a,X}$	$1.24\pm0.04^{a,X}$
Resilience (Ratio)	**Control**	0.003 ± 0.001^{X}	0.001 ± 0.000^{Y}	$0.005\pm0.000^{a,X}$	$0.001\pm0.000^{a,Y}$	$0.010\pm0.003^{a,X}$	$0.000\pm0.000^{a,Y}$
	OLE			$0.008\pm0.003^{a,X}$	$0.001\pm0.000^{a,Y}$	$0.015\pm0.003^{a,X}$	$0.000\pm0.000^{a,Y}$
Chewiness (N)	**Control**	1.15 ± 0.04^{X}	1.07 ± 0.10^{X}	$0.98\pm0.07^{a,X}$	$1.01\pm0.01^{a,X}$	$0.91\pm0.02^{a,X}$	$0.90\pm0.11^{a,X}$
	OLE			$1.10\pm0.04^{a,X}$	$1.13\pm0.09^{a,X}$	$0.95\pm0.02^{a,X}$	$0.93\pm0.03^{a,Y}$

Values are expressed as means ±SD, (n =3), W: white muscle, D: dark muscle.
a,b Different lowercase letters in the same column indicate significant differences ($P < 0.05$) between samples in the same muscle.
X,Y Different capital letters in the same row indicate significant difference ($P < 0.05$) between two muscles in the same sample

Table 3. Effect of OLE on texture profile analysis in white and dark muscles of vacuum-packed frigate mackerel stored at a refrigerated temperature (3±1°C). (continued).

Storage time (days)		**12**		**15**		**18**	
Parameters	**Samples**	**W**	**D**	**W**	**D**	**W**	**D**
Hardness (N)	**Control**	$38.76\pm0.28^{b,X}$	$37.56\pm0.50^{b,Y}$	$35.29\pm0.26^{b,X}$	$33.28\pm0.14^{b,Y}$	$30.28\pm0.24^{b,X}$	$28.05\pm0.21^{b,Y}$
	OLE	$42.78\pm0.20^{a,X}$	$39.72\pm0.62^{a,Y}$	$40.97\pm0.07^{a,X}$	$37.12\pm0.22^{a,Y}$	$32.60\pm0.16^{a,X}$	$30.11\pm0.06^{a,Y}$
Springiness (Ratio)	**Control**	$1.62\pm0.05^{a,X}$	$1.67\pm0.02^{a,X}$	$1.53\pm0.03^{b,X}$	$1.63\pm0.07^{a,X}$	$1.42\pm0.04^{b,Y}$	$1.57\pm0.02^{a,X}$
	OLE	$1.70\pm0.10^{a,X}$	$1.66\pm0.03^{a,X}$	$1.70\pm0.01^{a,X}$	$1.66\pm0.03^{a,X}$	$1.52\pm0.04^{a,X}$	$1.54\pm0.03^{a,X}$
Adhesiveness (mJ)	**Control**	$1.07\pm0.05^{a,X}$	$1.13\pm0.04^{b,X}$	$1.03\pm0.05^{b,X}$	$1.07\pm0.02^{b,X}$	$0.90\pm0.10^{a,X}$	$1.05\pm0.04^{a,X}$
	OLE	$1.13\pm0.04^{a,Y}$	$1.21\pm0.02^{a,X}$	$1.16\pm0.03^{a,X}$	$1.22\pm0.03^{a,X}$	$1.10\pm0.01^{b,Y}$	$1.14\pm0.03^{b,X}$
Resilience (Ratio)	**Control**	$0.001\pm0.001^{a,X}$	$0.001\pm0.000^{a,X}$	$0.002\pm0.001^{a,X}$	$0.000\pm0.000^{a,Y}$	$0.003\pm0.002^{a,X}$	$0.000\pm0.000^{a,Y}$
	OLE	$0.004\pm0.003^{a,X}$	$0.001\pm0.001^{a,X}$	$0.006\pm0.003^{a,X}$	$0.001\pm0.001^{a,Y}$	$0.002\pm0.001^{a,X}$	$0.000\pm0.000^{a,Y}$
Chewiness (N)	**Control**	$0.80\pm0.01^{a,X}$	$0.73\pm0.06^{a,X}$	$0.75\pm0.13^{a,X}$	$0.64\pm0.02^{a,X}$	$0.62\pm0.03^{a,X}$	$0.56\pm0.04^{a,X}$
	OLE	$0.84\pm0.03^{a,X}$	$0.78\pm0.07^{a,X}$	$0.81\pm0.09^{a,X}$	$0.73\pm0.05^{a,X}$	$0.63\pm0.04^{a,X}$	$0.59\pm0.05^{a,X}$

Values are expressed as means ±SD, (n =3), W: white muscle, D: dark muscle.
a,b Different lowercase letters in the same column indicate significant differences ($P < 0.05$) between samples in the same muscle.
X,Y Different capital letters in the same row indicate significant difference ($P < 0.05$) between two muscles in the same sample

Springiness is an important characteristic of fish muscles. Initially, the springiness value of dark muscles was significantly higher ($p<0.05$) than the white muscles. It decreased in all samples during storage; however, no significant differences ($p>0.05$) were observed in the muscles. Regarding the individual muscle type, the differences in the springiness values were not significant ($p>0.05$) between the treatments, except at day 4, 15 and 18 where springiness

values of white muscle from the treated group were significantly higher ($p<0.05$) than control. These findings confirm that OLE mainly improves the springiness of white muscles only.

Adhesiveness of both dark and white muscles from control groups was significantly ($p<0.05$) reduced until day 4 and of storage, respectively, followed by a sharp increase on the next sampling day. The similar tendency was also observed for both muscles in the treated group. The increase in adhesiveness might be due to the microbial growth resulting in protein degradation (Lopez-Caballero et al., 2005). Significant differences ($p<0.05$) in adhesiveness were witnessed between the treatments in both muscles, and it was significantly higher ($p<0.05$) than their corresponding controls. In the control samples, the adhesiveness of dark muscles was significantly higher ($p<0.05$) than that of white muscle at the early stage of storage (until day 8). For the remaining storage period, no significant differences ($p>0.05$) were observed between both muscle types. However, for the treated groups, dark muscles had significantly higher ($p<0.05$) adhesiveness than white muscles only on day 4, 12, and 18 of storage. The final values of adhesiveness were largely superior to the initial ones. Thus, the OLE and VP combination seemingly improved the adhesiveness of frigate mackerel muscles.

The elasticity of white muscles given in resilience was significantly higher ($p<0.05$) than the dark muscles throughout the investigation. Irrespective of muscle type, the changes in the resilience over a period of time were not significant ($p>0.05$) between both treatments. Thus, the application of OLE along with VP had no major effect on the resilience of both muscles.

Chewiness was also affected in all samples and diminished during the storage. No significant changes ($p>0.05$) were observed in the chewiness between both muscle types throughout the study. The treatment had no statistically marked effect on chewiness of the muscles, showing that the combined application of OLE and VP did not improve the chewiness of the muscles.

Consequently, the combined application of OLE and VP improved the textural attributes of both frigate mackerel muscles by affecting only the hardness, springiness, and adhesiveness.

4. Conclusions

The sensory data revealed that the shelf-life of both frigate mackerel muscles was found to be 12 days for the control samples, while OLE extended the shelf-life of frigate mackerel fillets by 3 and 6 days in white and dark muscles, respectively. The application of 0.5% OLE with VP apparently initiated the lipid oxidation at the earlier stage of storage and was less effective in reducing microbial growth in both muscles. However, the combined application of OLE and VP improved the texture parameters and exhibited the major impact only on hardness, adhesiveness, and springiness of both frigate mackerel muscles. Therefore, in order to make this system commercially viable, further research work is needed by employing different strategies such as the improvement of a plant extraction method for the recovery of more phenolic compounds, direct application of the extract on fresh seafood products or exploring other extracts with higher lipid solubility.

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EL KADID A TRADITIONAL SALTED DRIED CAMEL MEAT FROM ALGERIA: CONTRIBUTION TO THE STUDY OF THE COMPOSITION IN BIOGENIC AMINE, ORGANIC ACID, AROMATIC PROFILE AND MICROBIAL BIODIVERSITY

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ABSTRACT

Many meat preparations are manufactured in North Africa.in Algeria, the dried salted camel meat is called El kadid. The first analysis of the composition of biogenic amine, organic acid and aromatic profile of this traditional meat was carried out in this study. El kadid is obtained by salting, maceration and drying of camel meat. The results showed that the product contains lactic acid (6.22 g / kg), acetic acid (<1 g / kg). Biogenic amines are present at very low concentrations (<20 mg / kg). The aromatic profile highlighted the presence of more than 60 molecules belonging to different chemical classes, such as aldehydes, ketones and alcohols. El Kadid presente has a rich microbiological niche (Enterobacteriaceae Latic acid bateria Coagulase negative microstaphylococci yeast, Enterococci and *E coli*).

1.Introduction

The breeding of large camelids remains marginal on a global scale: 0.4% of the world's herbivorepopulation, 1.4% of the domestic herbivore biomass (HDB) (Faye, 2009**)**. According to the FAO, camelmeat production in Algeria will increase from 4800 Tons in 2010 to 5948 Tons in 2017.The importance of camels as meat-producing animals is gradually increasing due to the high nutritional value of their meat, in addition to their ability to survive in difficult environmental conditions. The composition of camel meat is similar to that of meat from other species where the inverse relationship exists between humidity and protein and fat content. In addition to its importance in determining the nutritional value of the meat, it is also an important indicator of the functionality of the meat (Bekhit and Farouk, 2013). Meat in particular is a very favourable breeding ground for most microbial contaminations. It is a difficult food to preserve. The shelf life of this product depends on the degree of acidity and water content of the product as well as certain external parameters such as air, micro-organisms, temperature and light (Berkel et al., 2004; Pal et al., 2018). There are many methods of meat preservation based on different principles: conventional methods and potential methods (Prokopov and Tanchev, 2004). Due to the high ambient temperatures and lack of refrigeration capacity most areas where camel meat is produced involve the use of artisanal curing technology (Kadim et al., 2013). Large

amounts of meat products are customarily prepared for the Eid-ul-Adha festival in Algeria; the excess has to be preserved for later consumption (Bouchefra et al., 2019). El kadid, a typical product of the Maghreb region (Morocco, Algeria and Tunisia), is obtained using this basic technology to produce stable salted cured meat that can be stored at room temperature for more than a year (Touzi and Merzaia-Blama,2008; Benkerroum, 2013). Unfortunately, there is no data on the composition and microbial biodiversity of dried salted camel meat, *El Kadid*, produced in Algeria. In this context, this study focuses on the traditional process of preparation of camelin cured salted dried meat (El Kadid) and an analysis of the composition of biogenic amines, organic acid and aromatic profile of El kadid.

2. Materials and methods

2.1. Traditional process for the preparation of salted dried meat (El Kadid)

The sample analyzed is a typical product of the Algerian terroir based on dried salted camel meat known as El Kadid. The camel meat is washed under running water and then cut into strips (approximately 1 cm wide and 10-20 cm long). Then other ingredients such as coarse salt, pepper and olive oil are added to the strips of meat. The meat is left to macerate overnight at 4°C. In the morning, we proceeded to the drying phase, during which the strips of meat are dried on ropes under the sun for about 7 days. During the drying period, the meat is turned periodically, in order to obtain an even drying of the final product. The drying phase ends when the meat reaches a brown colour and a good fibrous appearance. Finally, the strips of meat are cut into 2 to 3 cm long pieces and placed inside a sterile jar, sealed and kept at room temperature (Bouchefra et al., 2019).

2.2. Analysis of biogenic amine content

The qualitative-quantitative analysis of the biogenic amines was carried out by the technique of high-pressure liquid chromatography (HPLC). The first extraction phase was carried out as follows: 10 g of the El kadid sample were weighed, to which 20 ml of an aqueous solution of trichloroacetic acid (TCA 5%) (Sigma-Aldrich, St Louis, MO, U.S.A.) was added. The samples are then left for 30 minutes in a thermostatically controlled bath without stirring at 75°C. The extract is then centrifuged (Rotor JA 25: 50, 2700 rpm, 10 minutes, 4 °C) and filtered on filter paper into a 50 ml bottle. The extracts were derived according to the method described by (Martuscelli et al., 2001) and then injected into (a Jasco PU-2089 Plus HPLC system with a Rheodyne manual injector with 20 µl loops and a Jasco UV-VIS UV 2070 Plus UV detector at 254 nm). For the analysis, we used a C18 reverse phase chromatographic column (Waters Spherisorb ODS2, 150x4.6 mm, 3 uM) with the pre-column (Waters Spherisorb S5 ODS2, 4.6x10mm). The quantification of the amines occurs by using external calibration curves, obtained with amines from standard solutions (histamine, 2-phenylethylamine, tyramine, putrescine, cadaverine, spermine and spermidine) at different concentrations (10, 20, 30, 50, 75 and 100 ppm) which are subjected to the same derivatization procedure.

2.3. Analysis of organic acid content

The determination of the organic acids was carried out by HPLC injection of the El kadid sample, following an extraction treatment. 20 ml of 0.01N H2SO4 solution was added to 10 g of the sample. The solution was left under stirring at 30°C for 30 minutes. The supernatant was filtered through Whatman No. 1 paper filters into 10 ml bottles and brought to volume (50 ml) with the same extraction acid. The resulting sample was injected into a (Jasco PU-2089 Plus HPLC system with a manual injector model Rheodyne with a loop from 20µl). For the analysis we used an Aminex Bio-Rad chromatographic column (Bio-130 Rad Laboratories, Hertfordshire, UK) HPX-H (300x7.8 mm), maintained in an oven at 65°C. The mobile phase is characterized by the 0.005M H2SO4 eluent at a flow rate of 0.600 ml/min. The detector used is of UV type and the analysis is performed at 210 nm. The

chromatographic peaks were compared with the chromatogram obtained by injection of standard solutions of organic acids of interest and thus identified by comparison with the retention time of standard solutions of different organic acids thanks to retention times. The quantification of the organic acids is done by using external calibration curves, obtained with standard acid solutions (lactic acid and acetic acid) at different concentrations (Montanari et al., 2014).

2.4. Analysis of the aromatic profile

The aromatic profile of El Kadid was determined by the technique of gas chromatography-mass spectrometry coupled with solid phase microextraction (SPME-GC-MS) according to the method of (Tabanelli et al.,2015) :Three g of El Kadid were placed in a sterilized vial, sealed by PTFE / silicone partitions and heated at 47°C for 50 min. Volatile compounds were adsorbed for 40 min on a fused silica fiber covered with 75 mm Carboxen Polydimethyl Siloxane (CAR / PDMS Stable Flex) (Supelco, Steinheim, Germany). The adsorbed molecules were desorbed in the gas chromatograph for 10 min. For peak detection, an Agilent Hewlette Packard 6890 GC gas chromatograph equipped with a 5970 MSD MS detector (HewlettePackard, Geneva, Switzerland) and a Varian Chrompack CP Wax 52 CB capillary column (50 m× 320 µm□~ 1.2 µm), fused silica capillary column (Chrompack, Middelburg, the Netherlands) as the stationary phase were used. The injection conditions were: injection temperature, 250 °C; detector temperature, 220 °C; carrier gas flow rate (He), 1 ml/min. The furnace temperature was programmed as follows: 50°C for 1 min; from50°C to 65°C, at 4.5°C / min; from 65°C to 230°C, at 10°C / min, then holding for 25 min.Identification of volatile peaks was performed by computer matching of mass spectral data with those of compounds contained in the Agilent Hewlette Packard NIST 98 and Wiley Version 6 mass spectral database.

2.5. Mirobial biodiversity analysis

In order to assess the biodiversity of the microflora present in El-kadid salt-dried meat, sampling was carried out on the surface, using different selective culture media according to the method described by (Tabanelli et al., 2012**):**

-MRS agar (De Man, Rogosa, Sharpe): used for the enumeration of lactic acid bacteria by the surface inoculation method. After inoculation, the plates were incubated at 30° for 48 hours. The medium is prepared with a formulation (Oxoid, Basingstoke, UK).

-Glose Baird Parker: used for the growth of Gram-positive bacteria (Microcaccaceae and Staphylococcaceae). The medium is prepared with a formulation (Oxoid, Basingstoke, UK), after sterilization an emulsion of egg yolk and tellurite is added (Oxoid, Basingstoke, UK), to promote colony growth. After inoculation, the boxes were incubated at 30° for 48 hours.

-Slanetz and Bartley medium: suitable for the enumeration of enterococci by surface seeding. The medium was obtained using a formulation (Oxoid, Basingstoke, UK). The boxes were incubated at 42°C for 48 hours.

-VRBGA medium (glucose red bile agar, oxoid): for the enumeration of Enterobacteriaceae. The medium is prepared using a formulation (Oxid, Basingstoke, UK). The boxes were incubated at 37°C for 24 hours.

- PCA medium (Plate Count Agar,Oxoid): for the 174 enumeration of the total aerobic mesophyllic flora by surface seeding. The medium was obtained by the use of a formulation (Oxoid, Basingstoke, UK). The boxes were incubated at 30°C for 48 hours.

- SAB medium (Sabouraud Dextrose Agar, Oxoid): for the enumeration of yeasts by surface seeding. The medium was obtained using a formulation (Oxoid, Basingstoke, UK). The medium is supplemented with 200 mg / L of chloramphenicol to inhibit bacterial growth. After inoculation, the boxes were incubated at 28°C for 72 hours.

-VRBLA medium (Violet Red Bile Agar, Oxoid), supplemented with (4-methylumbelifery-B-D glucuronide, Oxoid) is

used for the determination of *Escherichia coli*. The medium has been prepared using a formulation (Oxoid, Basingstoke, UK). Once inoculated, the boxes were incubated at 37°C for 24 hours and then observed with UV light at 366 nm to count the fluorescent colonies.

2.6. Data analysis

The results are presented as the mean value ± standard deviation (SD) of three experiments Microsoft Excel 2016 was used for the data analysis.

3.Results and discussions

The composition in biogenic amine, organic acid, aromatic profile and microbial biodiversity of El Kadid has not been documented before.
The results of biogenic amine and organic acid are reported in Table 1. Among the organic acids, lactic acid is present in large quantities (6.22 g / kg), while acetic acid is present in small quantities (<1 g / kg). The analysis of biogenic amines which are substances derived from microbial metabolism (especially enterobacteria and lactic acid bacteria) that may pose a risk to the health of the consumer, has shown that these molecules are present at very low concentrations (<20 mg / kg). Histamine, putrescine and cadaverine are likely to result from the activity of Enterobacteriaceae (also present in the finished product) but their concentration is nevertheless below the amounts that could cause damage to the human organism. The same can be said of tyramine, the main producers of which are 196 lactic acid bacteria and in particular enterococci.

Table 1. Values of Organic Components and Biogenic Amines of El Kadid.

Acide lactique (g/Kg)	6.22 (± 0.76)
Acide acétique (g/Kg)	0.58 (± 0.06)
Histamine (mg/Kg)	12.31 (± 3.44)
Tiramine(mg/Kg)	18.61 (± 5.33)
Putriscine(mg/Kg)	9.10 (± 1.43)
Cadavérine(mg/Kg)	15.22 (± 2.79)

Results are expressed as means ± standard deviation of three measurements.

Table 2. Microbiological counts Log (CFU/g) of El Kadid product.

Enterobacteriaceae	2,50 (± 0,17)
Escherichia coli	<1
Latic acid bateria	2,93 (± 0,10)
Coagulase-negative microstaphylococci	6,16(±0,24)
Enterococci	2,08 (± 0,11)
Yeast	1,67 (± 0,20)
Total mesophyllic flora	6,24 (± 0,37)

Results are expressed as means ± standard deviation of three measurements.

The aromatic profile highlighted the presence of more than 60 molecules (Figure 1) belonging to different chemical classes, such as aldehydes (e.g., hexanal, octanal, nonanal), ketones (acetoin, 2,3-octanedione) and alcohols (1-octanol, 1-octene-3-ol). Aldehydes were the dominant class, probably due to sun drying (high temperature, light) which promotes the oxidation of lipids (Figure 2). The organoleptic characteristics of fermented meat derive from the combination of lipolytic and proteolytic, oxidative and endogenous, bacterial or even fungal enzymatic activities. Micro and staphylococci are responsible for the production

of most of the fermented meat flavour compounds, through the metabolism of amino acids and the consequent decarboxylation of aldehydes. Amino acids, especially leucine, isoleucine and valine, are transformed into alcohols and aldehydes, compounds related to the final flavour of the product. Moulds and yeasts can also contribute to the characterisation of the flavour profile of meat, thanks to their proteolytic and lipolytic capacities. Lactate oxidation, amino acid conversion and lipid oxidation by these microorganisms can contribute to the sensory quality of the finished product (Ravyts et al., 2012).

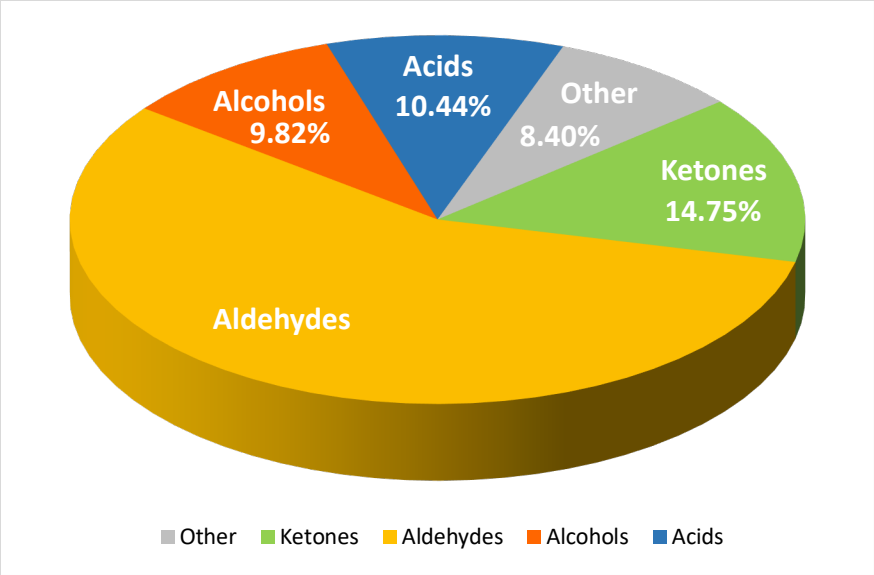

Figure 1. Volatile compounds grouped according to chemical classes (%) in El Kadid. Results are the mean of three samples. In bracket the standard deviation is reported.

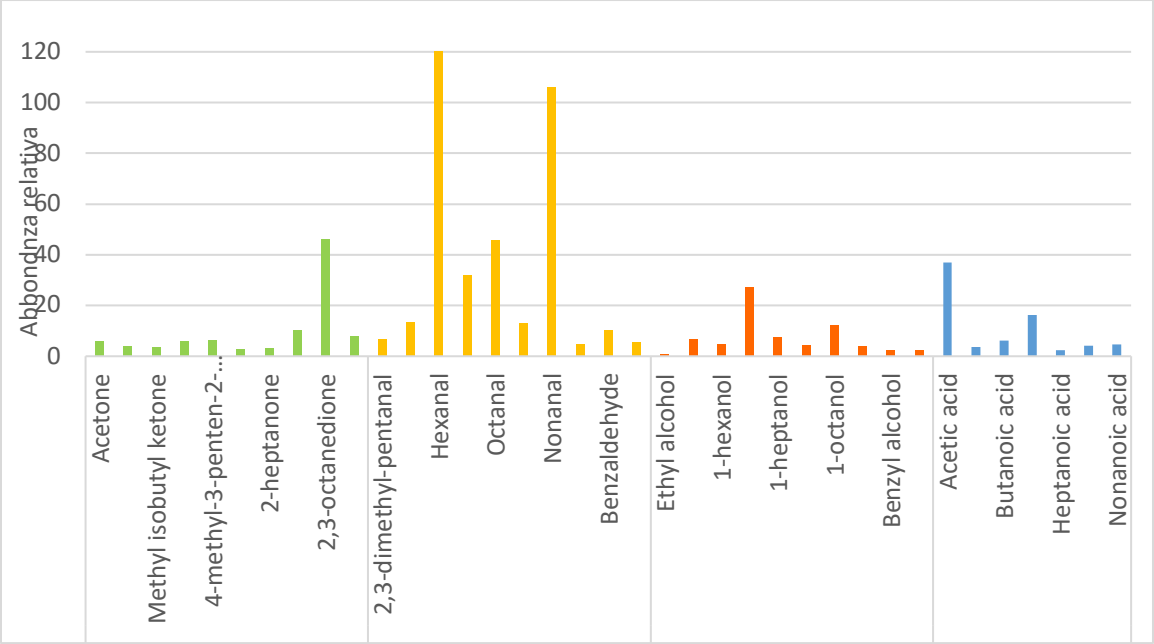

Figure 2. Aldehydes in El Kadid. Results are the mean of three samples.

The results of the microbiological counts, reported in Table 2, show that the dominant population is represented by coagulase negative microstaphylococci (CNC) (6.16 Log CFU/g) and yeasts, present at a concentration of 4.67 Log CFU/g. Other microbial groups are present at lower levels (2-3 Log CFU/g), in particular lactic bacteria, enterococci and enterobacteria. The latter probably derive from the handling of the product, which during the process is daily exposed to the sun during the day and then collected to be placed inside for the night. The value of the total mesophilic charge is almost superimposable with CNC counts. The microflora found is in line with what reported in the literature, where several authors have 218 highlighted staphylococci as the dominant population of this type of products (Bennani et al.,1995; Benanni et al.,2000).

4. Conclusions

The analysis of the composition of El kadid, a camel meat (*Camelus dromadarius)* obtained by drying after salting and marinating in olive oil typical of Algeria has shown that it contains small quantities of biogenic amines which does not constitute a health risk for the consumer. El kadid is characterized by a diversified aromatic profile which makes it appreciable by the tasters.Lactic acide bacteria are certainly the microorganisms of greatest interest from an industrial point of view and in particular for the selection of new starter and/or bioprotective cultures. The study of this product seems interesting to identify the risks associated with production and develop appropriate strategies to industrial level.

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QUALITY CHARACTERISTICS OF QUERCUS MACEDONICA, *Castanea sativa Mill.* VS *Quercus Alba* IN ORDER TO PRODUCE AN INNOVATIVE BALSAMIC VINEGAR PRODUCT

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ABSTRACT

Acetic fermentation is a vital function for microorganisms. Since antiquity, vinegar has been used both to dressing food, but also to characterize the irritable people. The aim of this study was to investigate the quality characteristics of Greek oak (*QuercusMacedonica* - QM) and Greek chestnut wood (*Castanea sativa Mill*. - CsM) in comparison with an American oak (*Quercus Alba* - QA) in order to produce vinegar from grape and apple. This study was conducted to make known the properties both of the Geek Macedonian Quercus and chestnut wood. All official OIV methods were used to implement this study. Volatile acidity increased in the apple vinegar more in QM and less in QA and CsM. In wine vinegar an increase in volatile acidity was observed at about the same level of 28 grams per liter expressed in acetic acid. It has therefore been observed that QM does not behave in the same way and rhythm as other forest species. We observe that QM does not release its ingredients quickly, so it is recommended for long aging of both wines and vinegars. QA and CsM release components from the first month. Polyphenols show a graduation from QM (about 1000 mg/lt) to CsM (approximately 15.500 mg/lt). All experimental data were confirmed by liquid chromatography. It was found that QM is best suited for aging apple vinegar. This study helps us to keep vinegar in every type of barrel, so to produce innovative balsamic vinegar.

1.Introduction

Acidic fermentation is responsible for sulfuric bacteria. These, through the enzymes contained in their cell, transfer oxygen to the alcohol to be oxidized until it is transformed into acetic acid. The history of vinegar is very great. The inhabitants of Attica in Greece called vinegar “idos” which mean pleasure or pleasant to taste. In ancient Lacedaemon (Greece), from the time of the king and legislator of Lykourgos in the 8th century BC century, people and army through the common soups eaten the famous «black broth» and gave them the reputation of prolific warriors. The Roman Empire set out an excellent cuisine in which the vinegar had a prominent place. Each house at the time of the Roman Empire had its own wine cellar (acetumlocum). The Romans used the vinegar to make “posca”, the drink of the legionnaires. After they first mixed the vinegar with water, they drank it in order to maintain their strength in the constant campaigns sent by the Roman Empire. Vinegar participates in many metabolic pathways of our organism. Finally, it has a place in local customs, especially in the Holy Week, perhaps because it is connected with the remembrance of the crucifixion and the offering of vinegar by the Roman soldier to the crucified Christ. Acetic acid is the main component of vinegar that plays an important role in the

release of energy from fats and carbohydrates. It also participates in the building of fats and amino acids. In the body it is transported with blood to the liver and tissues and undergoes complete oxidation with energy release (Kawa-Rygielska et al, 2018). In recent years there has been much talk about the balsamic vinegar of the Italian city of Modena, which is increasingly used in our kitchens. But long time is not the only thing that makes it so delicious and aromatic. It is aging in barrels made of wood of different species and in which vinegar is stored in succession. The maturation within them enriches it with a particular flavor. Polyphenols play an important role in giving flavour and special character to both wines and vinegars (Cerezo et al 2010, Del Alamo Sanza et al 2004, Sanzet al 2012a, Sanzet al2012b, Zhang et al 2015, Figueiredo-González et al 2014, Martínez-Gil et al 2019, Miriam Sanz et al 2011, Psarra et al 2015, Schwarz et al 2009, Tesfaye et al 2004, Bautista-Ortín et al 2008, Cerezo et al 2008, Cerezo et al 2010, Sarni et al 1990, Kanakaki et al 2015, De Rossoet al 2009, Sanz et al 2010, Alañón et al 2011, Karvela et al 2008). While acetic acid, in its pure form, is not so aromatic, it has a distinctly strong odor and irritates the olfactory centers when it comes into contact with them. Rapid induction of ageing character in brandy products is described many scientific projects (Van aarsveld et al 2009). Vinegars derived from sherry winesusually referred in bibliography (García Parrilla et al 1999, Alonso et al 2004). An innovative product of beer vinegar has been recently produced (Mudura et al 2018). In recent works, the chemical characteristics of the Greek CsM have been identified (Kakavas et al 2018, Chavenetidou et al 2019). In the present work we tried to produce an innovative vinegar product by dipping three different types of wood into wine and apple vinegar. It's the first attempt to use *Quercus Macedonica* for artificial aging of vinegars.

2. Materials and methods

2.1.Materials

2.2.1.Samples

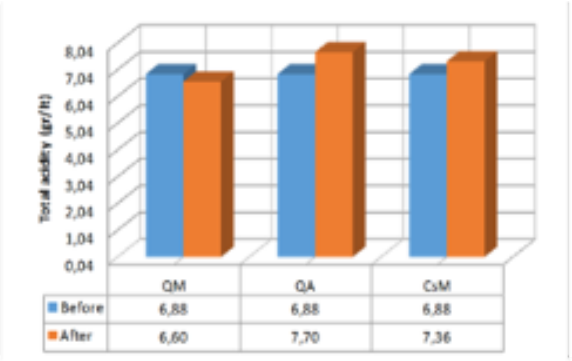
QM was given to us by the forestry authority of the Prefecture of Kozani and comes from Pindosmountain in Greece. The other types of wood and vinegars were purchased from the Greek market.We used glass bottles with a capacity of 300ml, in which 250ml of apple or wine (white) vinegar and 35gr of cubed wood were placed. Monthly analyzes were conducted for seven months.Total acidity (gr/lt tartaric acid), volatile acidity by Jaulmes (gr/lt acetic acid) and dry mass were (%) determined based on Hitos P. et al.Polyphenols were determined by the Folin-Ciocalteumethod: initially, the standard curve ($Y=0,0014X+0,0015$ $R^2=0,993$) was determined with various concentrations of gallic acid. Absorption was set at 765nm.Chromatograms data was performed with HPLC Agilent 1200.All official OIV methods were used to implement this study.

3.Results and discussions

3.1.Chemical Properties of Vinegar Samples

3.1.1. Total acidity

Total acidity has a declining trend for QM, with an increasing trend in QA and CsM before and after wood dipping in apple and wine vinegar (Figure 1). Total acidity was expressed in tartaric acid.



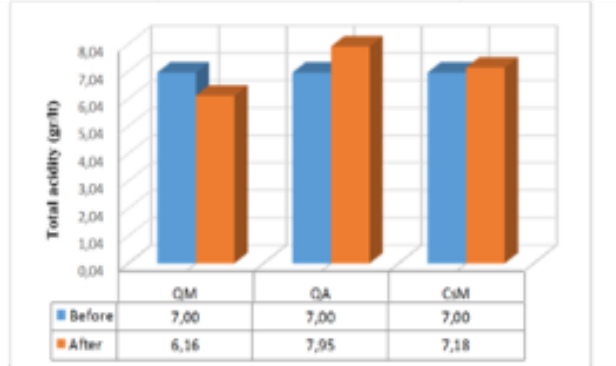


Figure 1. Total acidity of apple vinegar (left) and white vinegar (right).

3.1.2. Volatile acidity

Volatile acidity increased in the apple vinegar more in QM (28.7 gr/lt) and less in QA and CsM. In wine vinegar an increase in volatile acidity was observed more in CsM(29.1 gr/lt), (Figure 2). Volatile acidity expressed in acetic acid.

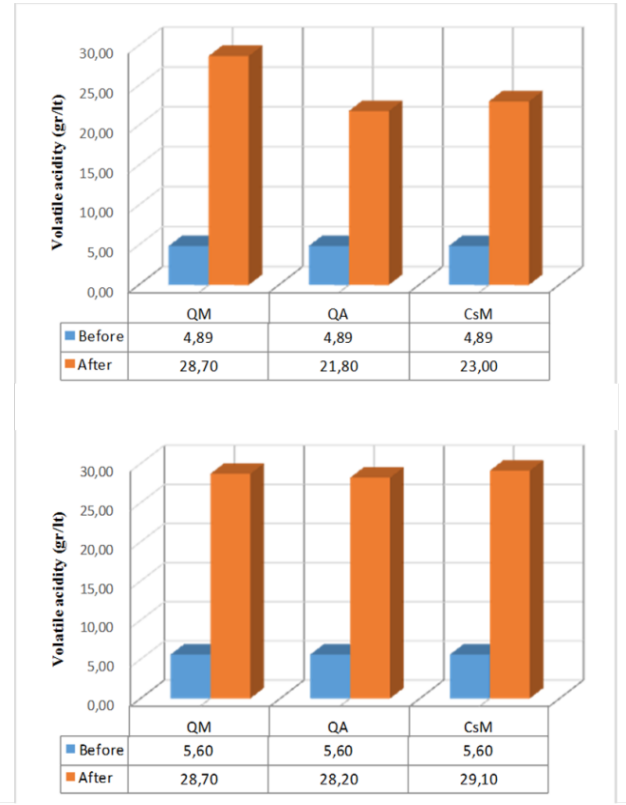
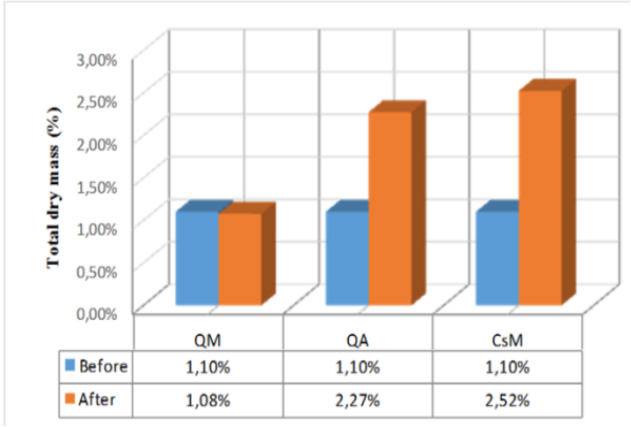


Figure 2. Volatile acidity of apple vinegar (left) and white vinegar (right).

3.1.3. Total dry mass

Total dry mass in both apple and wine vinegar is doubled in QA and CsM in relation to QM. It has therefore been observed that QM does not behave in thesame way and rhythm as other forest species (Figure 3).



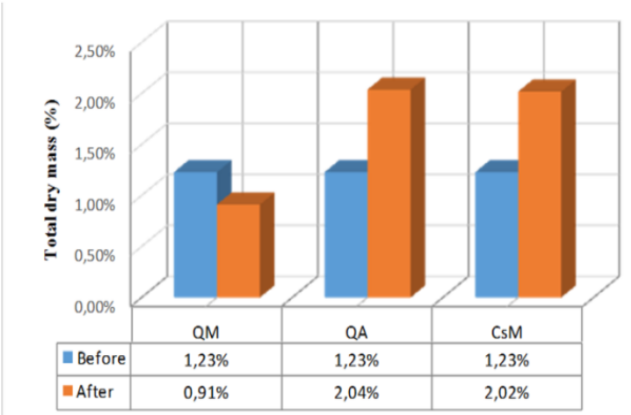


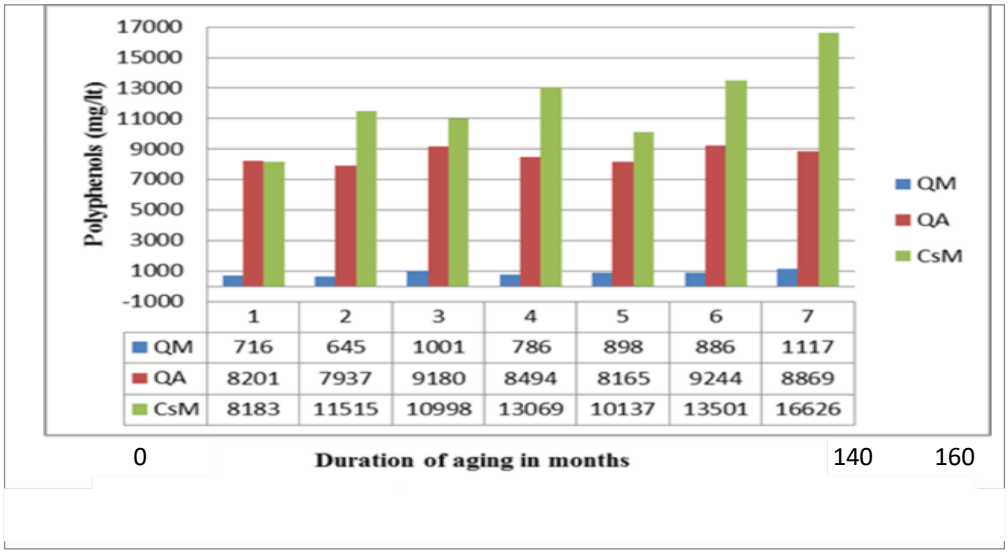
Figure 3. Total dry mass of apple vinegar (left) and white vinegar (right).

3.2.Biochemical Properties of Vinegar samples

3.2.1.Total polyphenols

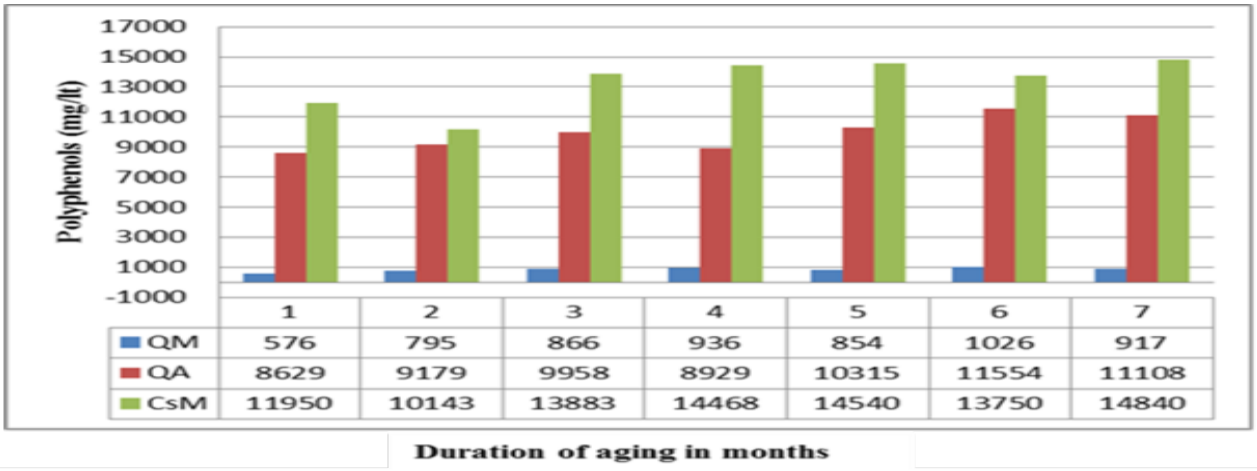
It is observed that CsM yields about 15,730 mg / lt, QA 9,990 mg / lt and QM 1020 mg / lt total polyphenols for seven months period, representing the average in the case of apple vinegar and grape vinegar (Figures 4, 5). We observe that among the three forest species, CsM yields most polyphenols in both apple vinegar and grape vinegar. In contrast, QM

yields fewer polyphenols. Polyphenols was expressed in gallic acid.



	1	2	3	4	5	6	7
QM	716	645	1001	786	898	886	1117
QA	8201	7937	9180	8494	8165	9244	8869
CsM	8183	11515	10998	13069	10137	13501	16626

Figure 4. Variation of polyphenols during aging in apple vinegar



	1	2	3	4	5	6	7
QM	576	795	866	936	854	1026	917
QA	8629	9179	9958	8929	10315	11554	11108
CsM	11950	10143	13883	14468	14540	13750	14840

Figure 5. Variation of polyphenols during aging in grape vinegar

3.2.2.HPLC chromatograms

If we compare the results of blank (wine vinegar) chromatogram with QM, QA and CsM samples (Figure 6 a - d) we see that, 6a chromatogram shows a limited number of peaks. 6b chromatogram shows all the peaks of the blind (6a) but in addition we can see peaks with deferent retention times. From 15 to 20, 22 to 29 and 53 to 56 minutes we can see many peaks. 6c and 6d chromatograms have a variety of peaks and verify that both QA and CsM have a large amount of polyphenols (see also Figure 4 and 5).

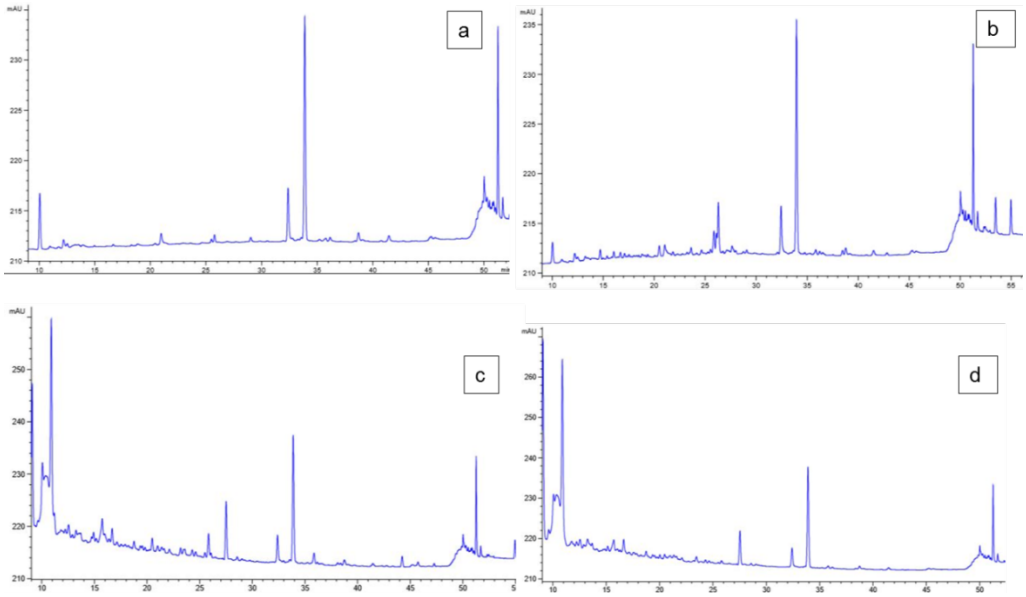


Figure 6.a) blank chromatogram (vinegar from wine), b) QM chromatogram, c) QA chromatogram, d) CsM chromatogram.

4. Conclusions

QM can create excellent balsamic vinegar from apples because the increase in volatile acidity has reached high levels(28.7 gr/lt). In contrast CsMcan create excellent balsamic vinegar from wine because the increase in volatile acidity has reached also very high levels(29.1 gr/lt).Total dry mass reached 2.52% in CsM and over doubled from its initial number (1.1%), in the case of apple vinegar. Total dry mass reached 2.04% toQA (2.02% to CsM) in the case of wine vinegar. CsMattributed more polyphenols to both apple vinegar (16.626 mg/lt)and wine vinegar (14.840 mg/lt). For this reason, CsM and QM can be used additionally in aging of wines and balsamic vinegars. This study helps us to keep vinegar the proper time period in every type of barrel, so to produce innovative balsamic vinegar.Our work revealed that Greek chestnut (CsM) showed much better quality characteristics than QA, in contrast to QM which did not yield rich quality characteristics.

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OPTIMIZATION OF RECIPE TURKEY MEAT PATE

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ABSTRACT

The article presents a study on optimization of the prescription of a paste made of Turkey meat. The technological scheme of production of pate, which determines the optimal operating parameters of industrial production of meat products, is presented. Five models of recipes for canned pates with a dietary orientation have been developed using mathematical modeling methods. Based on the results of the study of the biochemical composition and nutritional value, the optimal version of the recipe was selected, containing 31% of Turkey meat by weight. In addition to Turkey meat, the optimized recipe contains beef liver, fermented pork and chickpea flour. Qualitative characteristics of mechanical deboning of Turkey meat were studied. It is shown that Turkey meat meets the quality and safety requirements. The content of toxic elements in the studied batches did not exceed the acceptable values. The caloric content of Turkey meat pate is 226.8 kcal.

1.Introduction

The actual task of the meat and meat canning industry is to increase output and improve product quality by optimizing technological processes, identifying and using hidden reserves, and saving raw materials and energy resources. The range of products in the meat industry does not contain scientifically based recipes for canned meat products in the form of pates that meet the physiological standards of healthy nutrition.

Existing pate recipes are increasingly based on the possibility of using non-traditional raw materials and raw materials that can provide high quality. Turkey meat can be classified as dietary, and products made from it can be classified as delicatessen (Menon & Rumer, 2015).

The modern meat market is full of broiler meat. It is necessary to expand the range of meat products from poultry meat by increasing other types of poultry-ducks, geese, and especially turkeys (Bouvard et al., 2015; Prylipko & Kucius, 2014). Analysis of global trends in poultry farming shows a steady pattern of increasing Turkey production and consumption (Levy, Thaiss, & Elinav, 2016; Verkhovna Rada of Ukraine, 1997).

The development of science-based regimes that ensure guaranteed output of high-quality

products is impossible without an analytical description of the process, that is, a mathematical model of quality changes depending on changes in temperature and duration of heating of the product, the environment, the required and actual sterilizing effect. Recently, aseptic conservation is in high demand. This method is based on a new principle of thermal sterilization of food products (Alahakoon, Jayasena, Ramachandra, & Jo, 2015). The aseptic method of canning is one of the promising methods, but it has not yet been widely used in the practice of canning. When making canned food from products that have a uniform structure, you can use heat treatment of the product in the stream as it moves along the product pipeline (Menon & Rumer, 2015). The development of rational modes of sterilization of pates should be reduced not only to the study of the possibility of reducing the sterilizing effect when obtaining industrial sterile products, but also to establish the dependencies of objective criteria for quality indicators and nutritional value.

The task in different countriesis increasing the efficiency of using protein and fat meat resources available in this state for food purposes should be solved. The solution is possible by developing a new generation of recipes and creating original technologies for meat products with a guaranteed content of macronutrients and micronutrients. The relevance of this study is that meat pates are in great demand among customers, depending on the region of their production, are a product that has a fairly long shelf life, they are convenient to use, so they can be used in travel, for cooking various dishes. Undoubtedly, the task of quality control and safety of new pates produced, including Turkey meat, is important (De Mey et al., 2014; Decker & Park, 2010; Jiménez-Colmenero, Sánchez-Muniz, & Olmedilla-Alonso, 2010).

Based on the above, the goal of experimental research was formulated: to confirm the theoretical background of the process of sterilization of meat pate from Turkey meat, to optimize its composition and determine the optimal mode parameters of sterilization to preserve the quality characteristics of the original products, to evaluate the safety indicators of Turkey meat and finished pate.

The formulation and justification of composition was based on the following postulates: formulation of requirements for ingredients and product that meets the task at hand; the selection of ingredients to provide desired properties to the product; establishing acceptable levels (lowest and highest fractions) of the ingredient in the recipe; determining the optimal levels of ingredients in the recipe.

2.Materials and Methods

The research tasks included: studying the safety indicators of Turkey meat mechanical deboning used for the production of canned pates, conducting computer modeling of recipes with a new type of raw materials and the balance of main components to meet the requirements of a healthy diet, establishing the dependence of changes in the quality of pate on the level of thermal loads and justification of rational modes of sterilization of pates, selecting a new generation of canned pates technology and evaluating the prospects for implementing developments in the industry.

The object of the study was the meat of Turkey mechanically deboned. Experimental studies were conducted using generally accepted and standard methods used to study the physical, chemical and bacteriological parameters of meat products (Ibatullin et al., 2014).

The task of calculating the optimal recipe was formulated as follows: with the known lists of ingredients acceptable for the production of a particular product, and the characteristics of each of them (moisture content, fat, protein, amino acids, fatty acids, trace elements, cost, etc.), as well as the required mass of the resulting product, it is necessary to determine in what quantities it is advisable to include the ingredients in the recipe in order to meet the established requirements for chemical composition, the quality of the finished product and the amount of use of individual ingredients or their combinations, to ensure the minimum (maximum) value of the optimization criterion

(Ibatullin et al., 2017; Kabata-Pendias & Szteke, 2015).

The following steps were performed sequentially: data collection, systematization and analysis by characteristics of the selected list of ingredients and product requirements; calculation of optimal recipe options with the selected optimization criterion and set restrictions; verification of the obtained option for the possibility of improving the optimization criterion and the KZB and / or FSV and / or MEM; comparative analysis of the calculated recipe options and their selection for experimental verification (Waller et al., 2015).

5 recipes of Turkey pates were developed, from which the most optimal recipe was selected, characterized by the lowest complex indicator reflecting the dietary properties of the finished product.

The production technology of pate included the following sequential operations: preparation of raw Turkey meat, fermentation of pork skin, preparation of meat emulsion according to the recipe by successive introduction of ingredients of the recipe, placement of the emulsion in the packaging, sterilization, quality control of finished products, storage (figure 1).

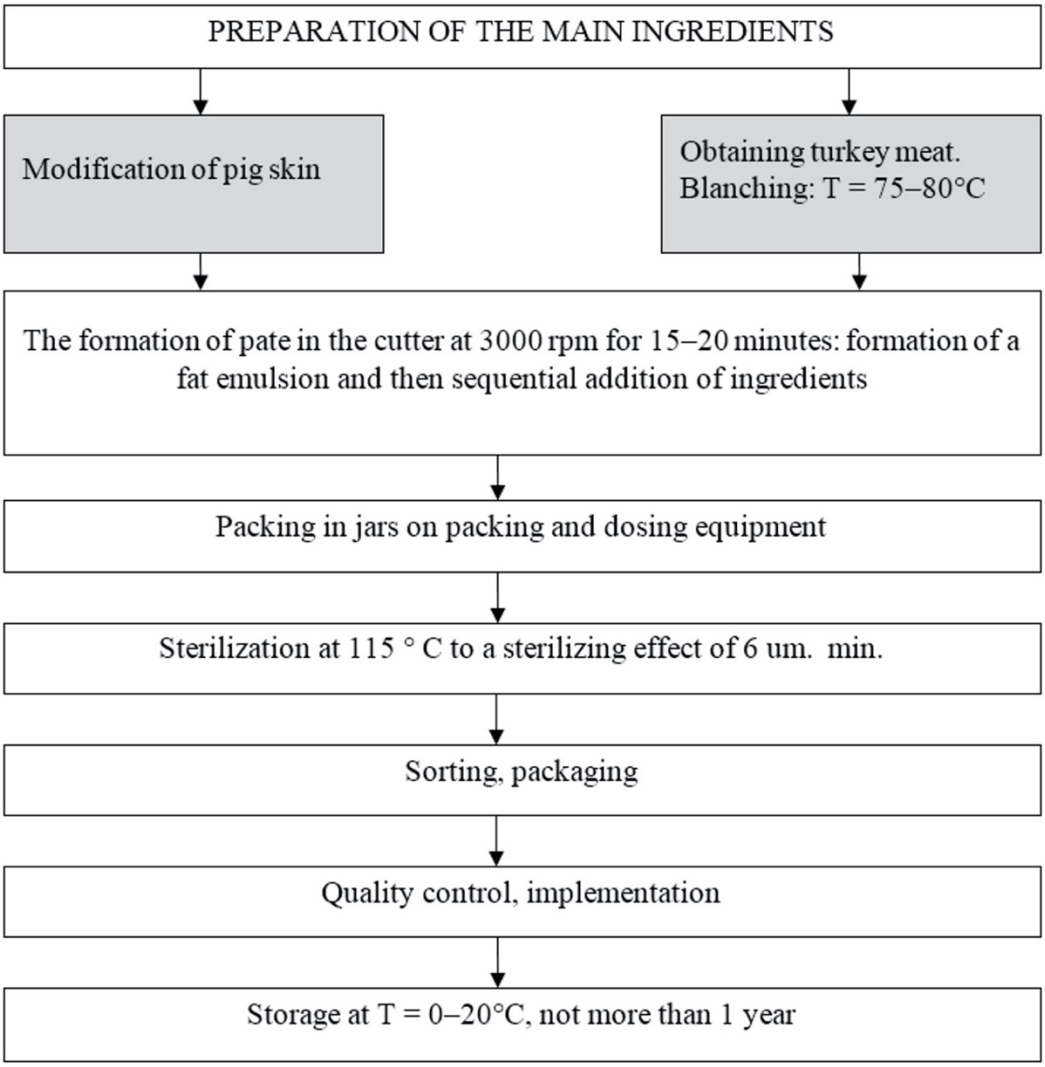

Figure 1. Flow Chart of Turkey Meat Pate Production

The content of toxic elements was determined by dry mineralization based on complete decomposition of organic substances by burning raw material samples in an electric

furnace under controlled thermal conditions (Ibatullin et al., 2014; Verkhovna Rada of Ukraine, 1997).

The quantitative content of mercury was determined by colorimetric method based on the decomposition of the sample with a mixture of nitric and sulfuric acids, followed by colorimetric determination of copper tetraiodomercurate in comparison with the standard scale (Prilipko and Kuchus, 2014; Verkhovna Rada of Ukraine, 1997).

The quantitative content of arsenic was determined by measuring the color intensity of a solution of a complex compound of arsenic with silver nitrate in chloroform (Bouvard et al., 2015; Prylipko And Kuchus, 2014).

The content of pesticides was carried out by extraction of organochlorine pesticides with organic solvents, purification of the extract, followed by analysis of the resulting solutions on an automatic gas chromatograph with an electron capture detector (on a column of chromatographic laboratory capacity of 35 cm with a partition of porous glass in the lower part) to identify the composition and determine the mass fraction of pesticides.

Standard values of toxic indicators (the content of antibiotics, toxic elements, pesticides of pathogenic microflora) were determined in accordance with current regulatory documents (technical regulation of the Customs Union No. 021/2011).

The proteolytic activity of enzyme preparations was studied using the Anson method using substrate hemoglobin.

Collagenase activity was determined by the content of oxyproline in the mixture formed as a result of the action of the enzyme on native collagen.

The active acidity (pH) was determined by a potentiometric method (Bouvard et al., 2015; Ibatullin et al., 2017).

The determination of the amount of mesophilic aerobic and optional anaerobic microorganisms was performed by counting colonies growing on solid nutrient medium after incubation at 30°C.

Sterilization regimens were determined by a method whereby the actual lethality of Ff relative to the microflora should be equal to or exceed the required lethality of the sterilization process Fn ($Ff \geq Fn$). The calculation of the required mortality was carried out on CL. sporogenes microorganisms. The required lethality was calculated by the formula:

$$F_н = D\,(4+\lg CG). \quad (1)$$

The experience was repeated five times. Statistical data processing was performed using the mathematical apparatus of the Excel program.

3.Results and discussions

3.1.Results

Regardless of the methods used, the formulation and justification of the formulations involves the following steps: formulation of requirements for the ingredients and product that meet the task; selection of ingredients that provide the desired product properties; setting acceptable levels (smallest and highest fractions) of the ingredient in the formulation; determining the optimal levels of ingredients in the recipe.

The task of calculating the optimal formulation was formulated as follows: with known lists of ingredients that are acceptable for the production of a particular product, and the characteristics of each of them (content of moisture, fat, protein, amino acids, fatty acids, trace elements, cost, etc.), as well as the required mass of the obtained of the product, it is necessary to determine in what quantities it is expedient to include the ingredients in the formulation in order to meet the established requirements for the chemical composition, quality of the finished product and the amount of use of the individual ingredients or combinations thereof, to provide the minimum (maximum) value of the optimization criterion (Ibatullin et al., 2017; Kabata-Pendias & Szteke, 2015).

The following steps were consistently performed: data collection, systematization and analysis according to the characteristics of the selected list of ingredients and product requirements; calculation of variants of the

optimal formulation with the selected optimization criterion and set restrictions; verification of the variant obtained for the possibility of improving the optimization criterion and KZB and / or FSW and / or MEM; comparative analysis of the calculated variants of the formulations and the choice of them for experimental verification (Waller et al., 2015).

We create a mathematical model for the calculation of raw materials for pate recipe. Denote x_1, x_2, x_3, x_4, x_5, x_6, x_7, x_8 as the amount of raw material of each species. F (x) is a target function that determines the content of ingredients. The mathematical interpretation of this problem will look like:

$$F(x) = 23{,}5x_1 + 24x_2 + 36{,}4x_3 + 92{,}3x_4 + 4{,}3x_5 + 10{,}3x_6 + 38{,}7x_7 + 10{,}8x_8 \rightarrow max \quad (2)$$

Based on the defined function and constraints, we create a spreadsheet in MS Excel according to functional dependency. The built-in Excel Solution Finder was used to determine the optimum composition of components.

For the most complete compliance of the product with the requirements of a healthy diet, it is necessary that the ratio of macronutrients in the product (proteins: fats: carbohydrates) is 1: 1.3: 4; the ratio of essential amino acids lysine: tryptophan: methionine is 1: 3: 3; the ratio of polyunsaturated: saturated: monounsaturated fatty acids is 1: 3: 6; the ratio of trace elements: Ca: Mg was 1: 1.5; Ca: P was 1: 0.6. The formulation was optimized to match these parameters.

The result of the mathematical analysis was the development of five virtual models of canned pate recipes (Table 1). A comprehensive evaluation of the formulations obtained showed that the fifth model is optimal, with a maximum value of this indicator of 3.0.

Table 1. Ingredients Composition and Characteristics of Virtual Recipe Models

Name of ingredients or indicators	**Virtual recipes models**				
	I	**II**	**III**	**IV**	**V**
Formulations. %					
Beef liver (X_6)	20.0	0	40.0	10.0	10
Turkey meat (X_1)	0	25.0	7.7	17.2	31.0
Fat smelted (X_3)	25.0	0	15.0	31.9	16.7
Sunflower oil	0	25.0	0	0.2	0
Fermented pork skin (X_2)	15.0	10.0	0	8.7	16.7
Beef brains (X_8)	10.0	10.0	12.0	0	0
Soy protein (X_4)	0	0	1.5	0	3.0
Chickpeas	0	3.0	0	1.5	0
Wheat fiber	0	0	0	1.0	0
Wheat flour (X_7)	5.0	2.0	4.0	2.5	0
Dried onions	1.0	1.0	0	1.0	0.8
Water added (X_5)	21.4	21.4	17.2	23.4	20.35
Nutritional supplements	1.5	1.5	1.5	1.5	0

Name of ingredients or indicators	Virtual recipes models				
	I	**II**	**III**	**IV**	**V**
Spices	0	0	0	0	0.35
Salt	1.1	1.1	1.1	1.1	1.1
Sugar	0	0	0	0	0
Together	100	100	100	100	100
Chemical composition. %					
Moisture	53.64	50.64	61.90	46.86	64.68
Fat	29.50	33.53	19.10	37.84	13.71
Protein	8.15	8.07	10.48	7.45	13.01
Including balanced	7.02	6.99	8.61	6.53	11.19
Carbohydrates	5.96	2.97	5.76	4.10	5.51
Ratio M: F: C	1:3.64:0.73	1:4.15:0.37	1:1.82:0.50	1:5.08:0.55	1:1.05:0.42
Amino acid composition. g					
Valine	0.404	0.348	0.692	0.324	0.664
Isoleucine	0.318	0.333	0.547	0.283	0.531
Leucine	0.557	0.584	0.953	0.499	0.931
Lysine	0.475	0.522	0.830	0.444	0.816
Methionine + cysteine	0.142	0.152	0.250	0.132	0.244
Threonine	0.283	0.272	0.476	0.232	0.460
Tryptophan	0.087	0.109	0.150	0.089	0.152
Phenylalanine + tyrosine	0.327	0.321	0.548	0.277	0.533
Minimum score. (methionine)	0.498	0.538	0.682	0.506	0.536
Balance factor	0.563	0.592	0.579	0.592	0.579
Correlation	1:0.3:0.18	1:0.29:0.21	1:0.3:0.18	1:0.3:0.2	1:0.30:0.19
Fatty acid composition. g					
Saturated fatty acids (H)	11.25	5.03	7.07	14.32	4.62
Monounsaturated fatty acids (M)	12.70	8.88	7.91	16.69	5.20
Polyunsaturated fat acids (P)	3.27	3.75	2.32	4.41	1.77
The ratio P: M: S	1:3.88:3.44	1:2.37:1.34	1:3.41:3.04	1:3.78:3.25	1:2.93:2.61

Name of ingredients or indicators	Virtual recipes models				
	I	**II**	**III**	**IV**	**V**
Or (P + M) / S	1.42	2.51	1.45	1.47	1.51
Trace element composition. mg					
Calcium (Ca)	4.4	11.5	12.2	7.7	12.7
Phosphorus (P)	123.8	95.1	204.7	77.7	185.4
Magnesium	7.9	12.1	16.3	9.6	16.8
The ratio of Ca: P: Mg	1:28:1.79	1:8.3:1.05	1:1.6:1.34	1:10:1.25	1:14.6:1.34
Estimates					
Calorie content. kcal	321.0	338.0	236.0	386.0	198.0
Relative metrics					
Protein balance	0.643	0.625	0.769	0.583	1.0
The ratio of unsaturated to saturated fatty acids	0.940	0.600	0.960	0.973	1.0
Calorie content	0.617	0.586	0.839	0.513	1.0
Comprehensive assessment of formulations	2.2	1.811	2.568	2.069	3.0

Based on the results of experimental studies, we have developed a recipe for meat pate with a dietary orientation. The selection of the recipe presented in the table below was based on a comprehensive assessment of such indicators as calorie content, protein balance, and the ratio of fatty acids (saturated and unsaturated) in order to obtain a dietary product. Recipe #5, which meets the above parameters, is shown in table 2.it is In this recipe that the complex assessment of the above parameters was the maximum (3.00), which corresponds to the requirements of the dietary product.

Table 2. The Best Recipe for Turkey Meat Pate

Name	Mass fraction of components. %
Turkey meat	31.000
Beef liver	10.000
Pork stud	16.700
Soy (flour)	3.000
Fermented pork skin	16.700
Fresh or dried onions	0.800
Salt	1.100
Black pepper ground	0.070
Coriander	0.070
Nutmeg	0.070
Carnation	0.070

Name	Mass fraction of components. %
Cinnamon	0.070
Water	20.350
Together	*100*

Studies on the content of antibiotics (levomycetin, tetracycline, bacitracin, grisin) have shown that Turkey meat, which is sent to the production of meat pate, does not contain antibiotic residues and can be used in production.

The results of studies to determine the content of pesticide residues in Turkey meat are shown in Table 3.

Table 3. Pesticide Content in Turkey Meat (M±m, n=5)

List of indicators	Laboratory analysis data	Requirements of the regulatory document
Hexachlorocyclohexane (alpha, beta, gamma isomers)	0.02±0.03 mg/kg	not more than 0.1 mg/kg
DDT and its metabolites	0.03±0.04 mg/kg	not more than 0.1 mg/kg

The quantitative analysis of the results of the experiments shows that the content of isomers of hexachlorocyclohexane in turkey meat is 0.02 mg/kg, which is 5 times ($P < 0.01$) below the acceptable values (0.1 mg/kg). DDT and its metabolites are 0.03 mg/kg, which is 3.3 times ($P < 0.01$) below normal.

Among the toxic elements, the most threatening to human health are four: lead, cadmium, arsenic and mercury, which are able to accumulate in the human body and cause diseases that manifest themselves gradually, without pronounced symptoms, and have high biological activity, oligodynamic, oligodynamic cumulative properties, the presence of specific, including remote, effects on the body. The results of the studies are shown in Table 4

Table 4. The Content of Toxic Elements in Turkey Meat (M±m, n=5)

List of indicators	Laboratory analysis data	Requirements of the regulatory document
Lead	0.08±0.02 mg/kg	not more than 0.5 mg/kg
Arsenic	0.02±0.03 mg/kg	not more than 0.1 mg/kg
Cadmium	not found	not more than 0.05 mg/kg
Mercury	not found	not more than 0.03 mg/kg

Studies were conducted on the content of toxic elements in Turkey meat. The research results showed that the content of toxic elements in the studied samples met the requirements and did not exceed the maximum permissible concentrations. The results showed the following: the quantitative content of lead was 0.08 mg / kg, at $P < 0.05$, which is 6.2 times less than the maximum permissible concentration, the arsenic content was 0.02 mg / kg at $P < 0.05$, which is 5 times less than the maximum permissible concentration. The presence of cadmium and mercury in the samples was not detected.

One of the main indicators of safety of the selected raw material is the presence of mesophilic aerobic and optional anaerobic microorganisms (MAFAnM) in it. The results of the studies are shown in Table 5.

Table 5. Results from Studies on the Detection of MAFanM Content in Turkey Meat Mesophilic Aerobic and Optional Anaerobic Microorganisms (colonies forming units per gram)

Name of pathogens	Laboratory analysis data	Requirements of the regulatory document
Mesophilic aerobic and optional anaerobic microorganisms	not found	not more than 1,0x10 KFU/г

According to Table 6, the highest percentage (31%) of canned pate is made from turkey meat. The specified meat raw material has undergone laboratory testing and meets the requirements of quality and safety regulations.

According to laboratory studies, the raw material found does not contain MAFanM colonies and is therefore safe for the production of meat products.

Thus, on the basis of complex analysis of turkey meat, we can conclude that the safety of meat raw materials for the production of pies of high quality and nutritional value.

Beef liver is included in the recipe in the amount of 10% of the total mass of raw materials and at its nutritional value complements the quality indicators of pate. In order to give a gentle texture to the pate, a fermented pork skin in the brine is put into the recipe.

In selecting the temperature and duration of heating of canned food come in the first place. From the fact that a properly established sterilization regime should ensure the microbiological stability of canned food. Sterilization regimes should ensure that the growth of microorganisms that are potentially harmful to human health is suppressed, as well as those that can cause damage to canned food during storage. It should be borne in mind that the heating should be as minimal as possible to ensure high organoleptic properties and nutritional value of the finished pate. Sterilization of turkey pate is carried out according to the formula:

$$(A – B – C) / T = (25–40–25) / 115. \quad (3)$$

The developed recipe for canned meat from Turkey meat was tested at the Kamianets-Podilsky meat processing plant (Ukraine). The manufactured test batch confirmed that the developed pates meet the quality and safety requirements for similar canned food.

According to the data of physicochemical studies (Table 6), the content of the main macronutrients in the studied samples was as follows: the content of the mass fraction of moisture was 66.8%at $P < 0.01$, fat-20.0%at $P < 0.01$, protein-8.2% at $P < 0.01$, ash-1.46% at $P < 0.01$, carbohydrates-3.5% at $P < 0.01$. These values correspond to the standards established in the relevant product regulations. The product turned out to be low-calorie, since its caloric content was 226.8 kcal.

Table 6. Results of Physicochemical Indices of Turkey Meat Pate

Indicators	Contents, %
Wet	66.8 ±0.5
Fat	20.0 ±0.25

Indicators	Contents, %
White	8.2 ±0.09
Ash	1.46 ±0.02
Carbohydrates	3.5 ±0.03
Calorie content, kcal	226.8 ±1.0

According to microbiological indicators (the content of mesophilic aerobic and facultative anaerobic microorganisms and mesophilic aerobic microorganisms), canned food meets the requirements of industrial sterility.

Studies were conducted on the safety indicators of ready-made Turkey pate.

The study of the quantitative content of toxic elements in samples of turkey meat pate (Table 7) showed that the mass concentration of toxic elements did not exceed the maximum permissible values. So, the lead content was 0.02 mg / kg (P <0,03), 30 times lower than the acceptable level of cadmium is 0.01 mg / kg (P <0.01), 30 times lower than the acceptable level of arsenic - 0,05 mg / kg (P <0.01), which is 20 times below the permissible level of mercury - 0.005 mg / kg (P <0.01), which is 20 times below the permissible level.

Table 7. Results of a Study of the Safety of Turkey Meat Pate (M±m, n=5)

Indexes	Content	Norm
Concentration of toxic elements		
Lead	0.02±0.5	not more than 0.6
Cadmium	0.01±0.3	not more than 0.3
Arsenic	0.05±0.25	not more than 1.0
Mercury	0.005±0.5	not more than 0.1
Organochlorine pesticides		
HCG (gamma-isomer)	0.04±0.5	not more than 0.1
DDT and its metabolites	0.03±0.3	not more than 0.1
Antibiotics, units/g		
Tetracycline group	not found	not more than 0.01
Levomycetin	not found	not more than 0.01
Bacitracin	not found	not more than 0.02
Grisin	not found	not more than 0.5
Radionuclides, Bq/kg		
Cs-137	not found	not more than 180
Sr-90	not found	not more than 80

The content of organochlorine pesticides did not exceed the norm and corresponded to the quantitative value: for the gamma-isomer of HCG - 0.04 mg/kg, which is 2.5 times less than the maximum permissible value of 0.1 mg/kg, for DDT and its metabolites – 0.03 mg / kg , which is 3.3 times less than the maximum permissible value of 0.1 mg/kg.

The residues of antibiotics and radionuclides were not detected at all.

3.2.Discussions

Our analysis of the literature shows that it is currently particularly effective to produce new types of meat products that include multi-component emulsions based on new types of

meat raw materials (Alahakoon et al., 2015; Bouvard et al., 2015). From the analysis of literature sources, it follows that the development of recipes for pates from non-traditional raw materials and raw ingredients that can provide high quality canned pates is of practical interest. And such raw materials as Turkey meat can be attributed to dietary meat (Anufriev, Kolmykov, Emelyanov, & Zinina, 2016; Krishtafovich, Krishtafovich, & Sharafutdinova, 2014; Starikova, 2015).

In 2017, the volume of the world market for Turkey meat was 6305 thousand tons. The following year, there was a marked increase in the market against the background of increased consumption in the United States, Brazil, and Russia. According to the research by IndexBox Russia, the market has grown by +3.1% per year on average in wholesale prices over the past ten years and amounted to $ 13.2 billion. USA. The US remains the key Turkey-producing country in the world. At the beginning of the last decade, Canada became the second country in terms of Turkey meat production in America — 153 thousand tons per year (Alahakoon et al., 2015; Bouvard et al., 2015).

The calculations described above, performed using computer modeling methods, allowed us to develop recipes for preparing Turkey pates that meet the average daily needs. Our assessment of the nutritional adequacy of virtual pate models for all 5 recipes (the Recipes for all five pates are presented in Table 1) showed that the fifth model is optimal (table 2), with a maximum value of 3.0. This recipe, unlike other developed models, contains the largest amount of Turkey meat, while the fat content is minimized, the liver content is significantly reduced, and wheat flour is present. It was possible to balance the amino acid composition by introducing chickpea flour into the recipe. Chickpea proteins can replace animal proteins by their biological value, since they are a source of essential amino acids, in particular methionine, lysine and tryptophan. Due to the high protein content in chickpea flour, there are many purine bases. A number of authors suggest correcting the nutritional value of meat pates by introducing them with high-intensity nutritional components. For example, Baranenko D. A. and co-authors suggest using an enzyme preparation of proteolytic action, including chymotrypsin and pepsin in a ratio of 1: 1, to increase the nutritional value of connective tissue. At the same time, optimization of technological parameters of protein proteolysis allows to obtain a maximum value of 3.1 (Baranenko, 2013). Stepanova E. A. it is proposed to use paprika with herbs, garlic, cranberries, buckwheat flour in the production of pate, which allows you to get a product with a piquant taste (Stepanova, 2017). Esmat, Asuggest use pumpkin in the recipe of meat and vegetable pate based on horse meat and chicken liver. The proposed composition provides a product with dietary, hypoallergenic and antioxidant properties (Hassan, Hussein, & Hussein, 2013). Rui Xu developed pates with a protein product from melon seeds of reduced caloric content, enriched with an additional amount of trace elements and vitamins (Xu, 2012).

The use of strict input control allowed us to achieve high food safety of the final product . Thus, the studied safety indicators are both in raw Turkey meat (table 3-5) and in finished products (table 3-5). 7) meet the required parameters.

Turkey meat does not contain pesticides or antibiotics, which are regulated by law. The content of heavy metals is significantly lower than the normalized one. Due to the short term of fattening, Turkey meat is an environmentally safe raw material.

Our results are consistent with data from a number of domestic and foreign researchers who show that Turkey meat does not contain pesticides or antibiotics, since it is not used in poultry farming (Chernukha, Smetanina, Kuznetsova, & Lisitsyn, 2005; De Mey et al., 2014; Ibatullin et al., 2014, 2017; Jiménez-Colmenero et al., 2010, p.). This result is very important because it indicates that Turkey meat does not accumulate toxic compounds in high concentrations. The content of heavy metals is significantly lower than the normalized one. Our results are consistent with many studies on the bioconversion of toxic compounds from the environment into the muscle tissue of Turkey

meat (Baranenko, 2013; Chernukha et al., 2005; Levy et al., 2016; Prylipko & Kucius, 2014). Based on this data, we can recommend the use of Turkey meat for the production of pates in any region.

Based on the results of market research conducted in various countries, we believe that poultry meat and its processed products are socially important goods, and the volume of their production is a criterion for ensuring the country's food security. Pates have recently gained popularity among consumers as a ready-to-eat product with a high calorie content, as an alternative to meat and sausage products. The results of the evaluation of the nutritional value of Turkey meat pate showed a high content of protein (21.2%), carbohydrates (3.5%), trace elements (1.46%), and a balanced energy value of 226.8 kcal, which is confirmed by many studies (Anufriev et al., 2016; Bazhenova, Khamaganova, Pavlova, & Badmayeva, 2005; Krishtafovich et al., 2014). Therefore, poultry pates have a high biological value, and the business idea of producing high-quality meat pates is quite popular and financially attractive (Alahakoon et al., 2015; Chicken & Turkey Meat Production in Canada—Market Research Report, 2019; De Mey et al., 2014; Decker & Park, 2010).

Turkey meat is in stable demand in North America, Europe, and Latin America, but per capita consumption varies from country to country. Consumption of Turkey meat, like other types of meat, depends significantly on the population and disposable income, so countries with a high standard of living traditionally have a high consumption of Turkey per capita. In addition, Turkey meat is characterized by a seasonality factor – demand in the United States and Europe increases annually during the pre-holiday period, for example, in the run-up to Christmas (Chicken & Turkey Meat Production in Canada—Market Research Report, 2019; Kabata-Pendias & Szteke, 2015; Menon & Rumer, 2015).

The method of sterilization is widely used to increase the shelf life of meat products. A number of authors, including Kabata-Pendias & Szteke (2015), Ibatullin I. I. and co-authors (Ibatullin et al., 2014) believe that sterilization regimes in the production of pates smooth out differences between different types of prescription components, leveling the quality of canned food and eliminating the influence of raw material quality. However, we have shown that the sterilization of canned food using classical technology is the basis for ensuring not only high sanitary and hygienic indicators of their quality, but also preserving the individual composition and characteristics of the product. The proposed regime of thermal preservation guarantees the death of pathogenic and toxigenic microorganisms and microflora that cause spoilage of products.

The sterilization modes proposed in our work-at a temperature of 115°C-allowed us to obtain the necessary sterilizing effect. Thus, microbiological studies of Turkey meat have shown that Mafanm (CFU/g) is 1x102, which is three orders of magnitude lower than the requirements for raw materials. No pathogenic microorganisms were detected.

Due to the small size of the carcasses, their manual deboning is extremely time-consuming. Nevertheless, in the works of Decker E. A. and co-authors (Decker & Park, 2010), it is noted that the use of mechanical boning in their processing is of great interest. After sterilization, the quality indicators of the finished pate showed its high nutritional value. Values of indicators of nutritional and biological value, amino acid, fatty acid and mineral balance (data are presented in Table 1 and Table 6) allowed us to classify Turkey meat pates as functional products. According to our research, Turkey pate contains 25–27% dry matter, 21–22% protein and 2.5–4.0% fat. We agree with the authors who claim that the chemical composition of Turkey meat pate can be attributed to dietary products (Chicken & Turkey Meat Production in Canada—Market Research Report, 2019; Xu, 2012). Turkey meat is tastier and healthier than chicken, pork, and beef, and contains a large amount of vitamins and trace elements. Turkey meat is well absorbed by the body, its digestibility is 90%.

The combined analysis of all the data presented strongly suggests that Turkey meat

obtained as a result of mechanical deboning can be used in the production of pates.

Based on the research of functional, technological and microbiological parameters of meat, vegetable raw materials and the finished product, rational modes of production and storage of Turkey meat pates with guaranteed safety and high nutritional and biological value have been established.

The technological scheme for the production of Turkey pates for functional nutrition presented in figure 1 includes all the necessary operations for the preparation of raw materials and components of the recipe. The formation of the meat emulsion at a rotation speed of 3000 rpm should continue for 15-20 minutes, then the ingredients are added sequentially to the resulting fat emulsion. For the described parameters of the production process, it is recommended to store the finished product at T = 0°C-20°C, no more than 1 year.

Although Turkey is a much less common type of meat than chicken, there is expected to be a gradual increase in consumer interest in Turkey against the background of a growing trend in the popularity of "healthy" food in developed countries and increased attention to low-fat foods.

4.Conclusions

Based on the research of the biochemical composition and calculation of nutritional value, the choice of ingredients for cooking canned Turkey pate of a new generation was justified. As a result of mathematical analysis, five virtual models of canned pate recipes were developed. A comprehensive assessment of the obtained recipes showed that the recipe, in which the content of Turkey meat was 31%, used beef liver to give a tender consistency, which in its nutritional value complements the quality indicators of pate, as well as fermented pork, is optimal.

Qualitative characteristics of mechanical deboning of Turkey meat were studied. As a result, high protein content, low fat content and high iron content were found, which allows us to classify this raw material as a dietary material with a functional focus on the body of a vitamin and mineral complex.

To further optimize the recipe, it is necessary to continue research on changes in the qualitative and quantitative composition of the main food nutrients during the heat treatment process. This will allow you to get a dietary, but at the same time very nutritious product from environmentally safe and useful meat raw materials.

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COMPARISON OF SUPERCRITICAL FLUID AND SOLVENT EXTRACTION METHODS IN EXTRACTING BIOACTIVE COMPOUNDS AND MINOR COMPONENTS OF RICE BRAN OIL

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ABSTRACT

(Comparing supercritical fluid and Soxhlet extraction systems (SFE and SE) for concentrating bioactive components of rice bran oil was studied. SFE was utilized to fractionate high oryzanol (HO) and low oryzanol (LO) RBOs. The tocopherols, tocotrienols, oryzanols and ferulic acid were measured utilizing HPLC, while, phytosterols and fatty acids were quantified utilizing GC. The results demonstrated there was a significant difference ($p < 0.05$) in unsaponifiable of HO compared with LO and Soxhlet oils. In neutral lipids and phospholipids classes, there were no significant differences ($p < 0.05$) between the three oils. On the other hand, in glycolipids class, there was a noteworthy distinction in oil concentrated utilizing Soxhlet compared with SFE technique. There were distinctive concentrations of tocopherols, tocotrienols, and oryzanols separated by the two methods. Oryzanol in RBO demonstrated a significant difference ($p < 0.05$) between diverse fractions of RBOs. Consequently, extraction by SFE impacted the sum, composition, and antioxidant of lipid in RBOs.)

1.Introduction

(Rice bran oil (otherwise called rice bran extract) is the oil extracted from the germ and inner husk of rice. It is remarkable for its high smoke point of 213 °C and its gentle flavor, making it suitable for high-temperature cooking routines, for example, blend frying and deep frying. It is well known as a cooking oil in a few Asian nations, including Japan and China (Orthoefer, 2005), and can be concentrated by diverse techniques for extraction. Solvent extraction is generally utilized anyway; it is much of the time utilizing toxic solvents. Then again; these solvents have been recognized as an air contaminant since it can respond with different pollutants to create ozone and photochemical oxidants (Tanzi et al. 2012). These days supercritical fluid extraction (SFE) system offers a finer system for extraction as it is more safe and free from solvent contamination (Lang and Wai, 2001). There are a few preferences in utilizing the SFE as a part of oil extraction. According to Rozzi and Singh (2002), supercritical fluids have higher diffusion coefficients and lower viscosity than liquid solvents. The nonappearance of a significant surface tension permits the fast penetration of supercritical liquid into pores,

thereby serving to improve the extraction productivity. By controlling the temperature and pressure, SFE can influence the dissolvability of the extracting fluid and subsequent control of the shifting arrangement of component in a sample (Tanaka and Takeshi, 2004). Many researchers used SFE for extraction of unconventional oils (Mariod et al. 2010; Chan et al. 2009; Louli et al. 2004).

RBO comprise of around 3.0 to 5.0% of unsaponifiable matter, which contain a unique complex of naturally occurring antioxidant components, for example, tocopherols, tocotrienols and oryzanol (Lloyd et al. 2000). Unsaponifiable matter incorporates higher aliphatic alcohols, sterols, pigments and hydrocarbons that often discovered broke up in fats and oils, and that can't be saponified by caustic alkalis but are soluble in the extraction solvents (Guthalugu et al. 2006).

Gamma–oryzanol is a mixture of sterol esters of ferulic acid consists of ferulate ester of a mixture 4, 4-dimethyl sterols consisting of cycloartenol, 24-methylenecycloartanol, campesterol, Δ- stigmasterol, Δ- campestenol, campestenol, β- sitosterol, sitostenol and Δ-sitostenol (Ash et al. 2011). RBO also contain a variety of fatty acids such as oleic, linoleic and palmitic (Amarasinghe et al. 2009).

Xu and Godber (2000) compared solvent extraction of rice bran to supercritical carbon dioxide extraction at 50°C and 68.9 MPa pressure for the extraction of γ -oryzanol. From their study, they found that SFE extract may contain up to four times higher γ -oryzanol (5.39 mg/g of rice bran) in content relative to solvent extraction. Perretti et al. (2003) studied various conditions, particularly pressure and temperature to extract oil from the products and by products of rice to increase the concentration of natural antioxidants (tocopherol, tocotrienol and oryzanol) in oil. They applied high pressure extraction of 5000psi (4hr), 7500 psi (2hr) and 10000 psi and temperature from 40ºC, 60ºC and 80ºC, respectively. They further found that the extraction conducted at 10000psi and 80ºC gave the highest extraction yield.

Sarmento et al. (2006) demonstrated the precipitation of rice bran oil with distinctive concentrations of tocopherol and tocotrienol in the first and second separators by utilizing SFE. They found that, the ideal conditions for the extraction of parboiled rice oil enhanced with tocopherol and tocotrienol was at 200bar and 40°c. At this condition the yield of tocopherol and tocotrienol were much higher which were 234.96mg/100g and 704.16mg/100.

RBO contain a high measure of unsaponifiable matter that possibly can anticipate chronic illnesses Kahlon et al. (1996) proposed that the cholesterol lowering activity of rice bran may be impacted by the level of unsaponifiable matter in the lipid portions of rice bran. Oryzanol is a mixture of sterol esters of ferulic acid, which have physiological properties, for example, having superoxide dismutase-like antioxidant activity and hypocholesterolemic impacts in humans (Visser et al. 2000). This study aims to determine the composition of RBOs extracted using supercritical fluid extraction and Soxhlet systems, and to determine and compare the resultant lipid classes of SFE and Soxhlet RBOs.

2. Materialsandmethods

2.1.Materials

2.1.1.Samples

Rice bran was obtained from Bernas Rice Mills, Sg. Tiram, Selangor, Malaysia. The rice bran sample was stabilized using oven at higher temperature for 3 minute before the extraction commenced.

2.1.2. Chemicals

All solvents (chloroform, acetone, methanol, hexane, ethanol, hydrochloric acid, ethyl acetate, acetonenitrile, isopropanol, acetic acid, pyridine, bis trimethylsiyl trifluoro acitamide (BSTFA) and isooctane) were of analytical and HPLC grade obtained from Fisher Scientific (Loughborough, LE, UK) and Merck (Darmstad, Germany).

Chemicals such as potassium hydroxide, sodium chloride and sodium methoxide were obtained from Sigma - Aldrich (St. Louis, MO, USA). Standard for unsaponifiable matter (tocopherol, tocotrienol, oryzanol and phytosterol) and neutral lipid were purchased from Sigma - Aldrich (St. Louis, MO, USA). The FAME reference standard was purchased from AccuStandard (New Haven, CT, USA)

2.1.3. Instruments

Supercritical fluid extraction system (Thar Technologies, Pittsburgh, PA, USA model SF2000), rotary evaporator (Buchi, Flawil, Switzerland), centrifuge machine, universal and micro type (Hettich Zentrifugen, Tuttlingen, Germany), ultrasonic water bath (HanShin, USA), analytical balance (Shimadzu, Nakagyo Ku, Kyoto, Japan model number). Each component of the lipid in rice bran oil was determined using an Agilent Technologies HPLC series 1200 (Santa Clara, CA, USA) and Thermo Fisher Scientific Triple Quad GCMS (St. Waltham, MA, USA).

2.2. Methods

2.2.1. Extraction of RBOs

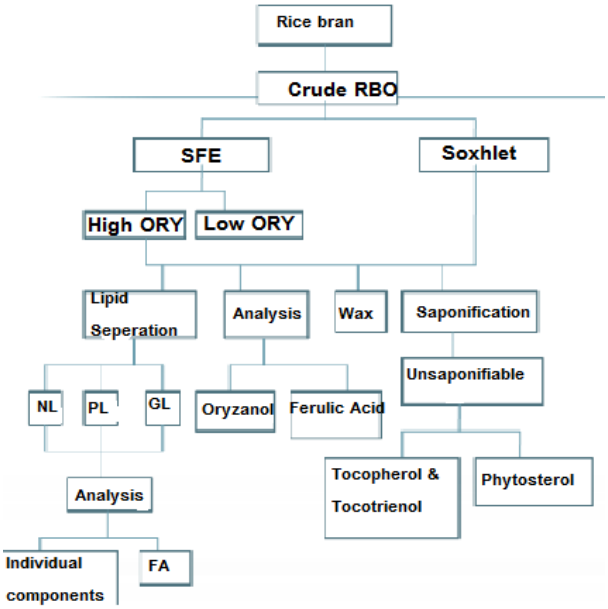

Figure 1. Layout of extracting bioactive compounds from rice bran using SFE and Soxhelt methods

2.2.2. SFE extraction

The high oryzanol RBO (HO) and low oryzanol RBO (LO) were concentrated by SFE utilizing distinctive weight and temperature (Fig.2). In a word, carbon dioxide (CO_2) from a cylinder was pressurized and fed into the solvent reservoir. This pressurized CO_2 was turned into the supercritical liquid when preheated in an equipped air-bath. After rice bran (180 g) was placed in the extractor, the supercritical liquid, then was streamed into the extractor and was blended with the rice bran. The extractor was joined with the collector vessel. The temperature and weight were controlled to obtain the SFE oil. After that the mixed solutions of RBO and supercritical liquid from the extractor were depressurized and the RBO was gathered (Kim et al. 2005). To get the LO from SFE weight of 600 bars and a temperature of 60°C were utilized. Meanwhile, to acquire the LO the pressure was 200 bars and temperature was 40°C. The flow rate and duration of time to concentrate HO and LO was comparative with 25g/min of flow rate and 150 mins duration time.

2.2.3. Soxhlet extraction

Utilizing the procedure described in the Official and Recommended Methods of AOCS (1998). Soxhlet extraction of RBO was done (Fig. 1). In short, 450 g of rice bran was weighed and put in extraction thimbles. The thimble was set in a Soxhlet extractor. Next, n-hexane was added into round flask, which had been joined with the extractor and condenser. Extraction methodology was begun when the solvent flow through the rice bran and extract the oil. After extraction was finished, n-hexane was removed under reduced pressure utilizing a rotary evaporator (Buchi, Flawil, Switzerland). Consequently, the Soxhlet RBO was set into a desiccator chamber for 1 h to absorb the moisture in the oil.

2.2.4. Saponification of rice bran oil

RBOs were saponified to get the unsaponifiable matter. This method is critical to examine sterol components in oils. 5.0g of RBOs sample was re-fluxed with 50 ml of

ethanolic potassium hydroxide (2m) for 1h. After the response, the sample was kept in overnight. At that point, the sample was mixed with 100 ml of distilled water to structure aqueous solution in a separation funnel. Next, the solution was washed with 100 ml of diethyl ether for 3 times this removes neutral lipid components. Hence the unsaponifiable matter was gotten after the removal of diethyl ether under diminished pressure (Canabate-Diaz et al. 2007). The weight and percentage of unsaponifiable matters were calculated

2.2.5. Separation of lipid classes

RBOs samples were separated according to the method described by Shin and Godber (1996). Column chromatography (25 mm i.d. X 30 cm) was used to separate the neutral lipid (NL), glycolipid (GL) and phospholipid (PL) fractions RBOs samples. A loading ratio of 3.0g oil: 30g of activated silica gel 70-230 Mesh (Merck, Darmstad, Germany) was used. NL was eluted first with 400 ml chloroform, GL with 600 ml acetone and PL with 400 ml methanol. Solvents were removed under reduced pressure and the yield of each fraction was determined gravimetrically.

2.2.6. Determination of wax

The waxy substance of RBOs was determined by utilizing the solvent dewaxing methodology. One gram of unrefined RBOs sample was weighed into a centrifuge tube, and one ml of chilled acetone was included in ratio 1:1. The solution (oil/acetone) was centrifuged at 4000rpm for 10 min in a universal centrifuge machine (Hettich Zentrifugen, Tuttlingen, Germany). The supernatant or the dewaxed oil was totally removed. The wax stayed at the base of the tube was washed with chilled actenone and centrifuged once more. The wax was gathered and the yield of wax was determined gravimetrically (Ramaswamy et al. 1980).

2.2.7. Analysis of neutral lipid (NL) of RBOs

The neutral lipid fraction that was collected from column chromatography was analyzed by HPLC using a C18 Supelco column (25 x 4.6mm, 5µ). Acetone/acetonitrile/methanol (50:50:10, v/v/v) was used as a mobile phase with an isocratic flow 0.5 ml/min. Column temperature was maintained at 30ºC and the components were detected using a refractive index detector (JASCO Model RI-2031, Easton, MD 21601, USA). Oleic acid, monolein, diolein and triolein were used as standards. The calibration curve of each standard was used to quantify the components such as monoaylglycerides (MAG), dicylglycerides (DAG), and triacyglycerides (TAG) and free fatty acid (FFA) in a neutral lipid fraction of RBOs samples (Shin and Godber, 1996).

2.2.8. Fatty acid analysis of RBOs

2.2.8.1 Preparation of fatty acid methyl ester (FAME)

An adjustment of the method depicted by Cert et al. (2000) was utilized. 25 mg of RBOs samples were weighed a test tube, and one ml of 0.2n of sodium methoxide in methanol was included. At that point, the example was warmed in a water bath (55ºC) for 20 minutes. After taken out from water bath, 1.0 ml of concentrated hydrochloric (HCl) acid was included and the incubation was continued in 40 minutes. Following 40 minutes the test tubes were taken out and 10 ml of 0.05m sodium chloride. The solution was mixed well and 2.5 ml of hexane were included. The upper layer of the solution was withdrawn and subjected to FAME analysis.

2.2.8.2. Analysis of fatty acid composition (FAME)

Analysis of FAME was carried out in the GCMS Triple Quad (St. Waltham, MA, USA) equipped with a splitless capillary inlet system with a TG-5MS column (15mm x 0.25mm id x 0.25µm, Agilent J&W DB-5ms Ultra Inert Capillary, Santa Clara CA 95051 USA) Others parameters involved in this analysis were as followed: injector temperature (250ºC); carrier gas (He); flow rate (1ml/min); oven temperature (50ºC hold for 1 minute until 280ºC and hold for another 5 minute). Fatty acid composition of RBOs was identified and quantified through the calibration curve of FAME standard

2.2.9. Analysis of unsaponifiable matters

Quantification of phytosterol

Sample preparation was carried out according to the method described by Toivo et al. (2000). The saponified RBOs sample was diluted with 10 ml pyridine. Then, 1.0 ppm concentration of RBOs samples was prepared by serial dilution. The sample was derivatized with a mixture of bistrimethylsiyl trifluoro acitamide (BSTFA) and isooctane (99:1). The sample solution was then heated at 60ºC before mixed with 500 µl hexane. Individual standards such as *β*-sitosterol, stigmasterol and stigmastanol were prepared using ethanol and used to determine the phytosterol content in the tested samples. Sterol components were separated using GCMS Triple Quad (St. Waltham, MA, USA) equipped with a splitless capillary inlet system with a TG-5MS column (15mm x 0.25mm id x 0.25µm). Others parameters involved in this analysis were as followed: injector temperature (250ºC); carrier gas (He); flow rate (1.0 ml/ min); oven temperature (50ºC and hold for 1 min until 300ºC and hold for another 5min. The sterol components of RBOs were quantified using the calibration curves for *β*-sitosterol, stigmasterol and stigmastanol standard.

2.2.10. Quantification of oryzanol by HPLC

The oryzanol content of RBO samples was analyzed as per technique created by Rogers et al. (1993) and utilizing reverse-phase HPLC. Oryzanol content was detected at 325 nm with Photodiode Array Detector (PDA). Oryzanols were differentiated with ODS (C18) Hypersil silica column (5µm X 250nm). The mobile phase comprised of acetonitrile/methanol/isopropanol with a ratio of 50:45:5 (v/v/v) and stream rate of 1 ml/ min. The sample was prepared by diluting it with ethanol before injection into the HPLC. A standard calibration curve of oryzanol standard was utilized to quantify the content of oryzanol in an oil sample.

2.2.11. Quantification of Ferulic acid

The evaluation of ferulic acid in RBOs samples was done as per method created by Zhou et al. (2004) and utilizing reversed phase HPLC. The components were differentiated by 2 ORBAX SB (C18) columns (150mm x 4.6mm, 5µm) and detected utilizing diode array detector at 280nm and with flow rate 1 ml/ min. The mobile phase was solvent A (water: acidic acid, 100:1, v/v) and solvent B (methanol: acetonitrile: acidic acids, 95:5:1.v/v/v). A standard calibration curve of ferulic acid standard was utilized to evaluate the content of ferulic acid in an oil sample.

2.2.12. Quantification of tocopherol

Tocopherols in RBOs were quantified utilizing an HPLC equipped with a UV detector and a 5 µm, 250 X 4.6 mm inertial NH_2 column. The mobile phase consisted of ethyl acetate/ hexane with ratio 30:70 (v/v). The flow rate was 1.0 ml/ min and increased slightly to 1.5 ml/ min for 5 min. The compound was at 290nm wavelength. The standard calibration curve of tocopherol standard was used to quantify the content of tocopherol in an oil sample.

2.2.13. Quantification of tocotrienol

Quantification of tocotrienol was completed as indicated by Shin and Godber (1994) technique and utilizing HPLC. Tocotrienol was detected utilizing fluorescence detector. Tocotrienol of the RBOs samples was detected at 330nm. A column that utilized in this system was Zorbax Nh2 (250 x 4.6 mm, 5µ) with a flow rate 0.5 ml/ min. The mobile phase comprised of hexane and isopropanol with proportion 99.5:0.5 (v:v). The standard calibration curve of tocotrienol standard was utilized to measure the substance of tocotrienol in oil test.

2.3.Statistical Analysis

The statistical analysis system SPSS 16 was used to perform statistical computations. Analysis of variance (ANOVA) accompanied using Tukey's multiple range tests and LSD were performed if a significant difference in the means of values at $p < 0.05$.

3.Results and discussions

TABLE 1. Lipid Composition of Rice Ban Oil Samples

		Sample (g/g)			
	Unsaponifiable matters	**Wax**	**Neutral Lipid**	**Glycolipid**	**Phospholipid**
HO	0.08±0.002[a]	0.17±0.10[a]	0.97 ±0.013[a]	0.03±0.01[a]	0.01±0.01[a]
LO	0.05±0.002[b]	0.18±0.001[a]	0.95±0.011[a]	0.03±0.000[a]	0.01±0.01[a]
Soxhlet	0.05±0.001[b]	0.16±0.063[a]	0.72±0.43[a]	0.01±0.003[b]	0.06±0.03[a]

a-b: The different letters within the same column indicate a significant difference ($p < 0.05$) Abb: HO – high oryzanol oil, LO – low oryzanol oil

3.1. Lipid composition of rice bran oils

Table 1 shows the estimation of lipid composition in RBO samples. This table showed that the estimated amount of unsaponifiable matter of Soxhlet RBO was about 0.048±0.001 g/g oil which represents about 4.8%. A previous study reported that the amount of unsaponifiable matter of crude rice bran oil was approximately 4.0% (Lloyd et al., 2000). The amount of unsaponifiable matter in HO (0.08±0.02 g/g oil) was estimated to be about two times higher ($p<0.05$) than the Soxhlet method (0.05±0.01 g/g oil). Meanwhile, the unsaponifiable matters in LO was 0.05±0.02 g. Extractions by SFE might have influenced the extraction composition in rice bran. As reported by Dunford and King (2000) fractionation of RBO using supercritical fluid carbon dioxide (SCC) can minimize the loss of unsaponifiable matter in samples. This is due to a closed SFE system that prevents oil from exposed to air. Exposure of RBOs to air for a longer time causes the degradation of antioxidant which is abundant in unsaponifiable matter (Duvernay et al. 2005). From Table 1, the wax content in three different types of oil extraction was different. The amount of wax estimated in HO and LO was 0.17±0.09 g/g oil and 0.18±0.01 g/g oil, respectively. Compared with Soxhlet RBO, amount of wax was 0.16±0.06 g/g oil slightly lower than RBOs extracted by SFE ($p\leq 0.05$). This was supported by Patel (2005) who found that RBO extracted using the SFE method was high in waxes.

As shown in Table 1, lipid classes in different types of oil extraction were different. In NL and PL classes, there were no significant differences in these 3 different types of extractions (HO, LO and Soxhlet). However, in glycolipid classes there was a significant difference in Soxhlet RBO compared with oil extracted using SFE. As shown in Table 1 the estimated amount of NL in HO and LO were about 0.97±0.01 g and 0.95±0.01g which was almost 90%. As expected, NL is a major lipid in RBO as showed by Shin and Godber (1996) where they found that amount of NL in RBO was 89.2%. Meanwhile, the estimated amount of neutral lipid in Soxhlet RBO was lower from RBO that extracted using SFE.

The estimated amount of neutral lipid in Soxhlet RBO was about 0.72 ±0.43g. Yet, there was no significant difference in amount of NL in each different type of oil extractions. Instead, the estimated amount of GL in Soxhlet RBO was lower than that of HO and LO which was 0.08±0.03 g ($p<0.05$). Meanwhile, the estimated amounts of GL in HO and LO were about 0.03±0.08 g and 0.03±0.000 g. The different extraction of RBO slightly gives different amounts of GL extraction. It seems that SFE method increased the amount of GL classes in rice bran oil. In addition, there were no significant differences in amount of PL in the different type of oil extraction. Amount of PL in HO and LO extracted using SFE were slightly similar which was estimated about

0.01±0.01 g and 0.01±0.01 g, respectively. However, the amount of phospholipids (0.06±0.03 g) in a Soxhlet extracted oil was significantly ($p<0.05$) higher.

3.1.1. Analysis of unsaponifiable matters of rice bran oils

Table 2. Compounds in Unsaponifiable Matters in Different Extract of Rice Bran Oil

Sample (mg/g)	a-Tocopherol	Tocotrienol	[x]Oryzanol	[y]Oryzanol	Ferulic acid	Phytosterol
HO	11.61±0.8[a]	2.62±0.2[a]	7.84±0.04[a]	ND	ND	ND
LO	6.47±0.1[a]	3.24±0.6[a]	6.17±0.02[b]	ND	ND	ND
Soxhlet	23.12±3.0[b]	2.45±0.1[a]	4.81±0.11[c]	ND	ND	ND

a-c: The different letters within the same column indicate significant difference ($p < 0.05$) Concentration of gamma-oryzanol in RBO samples with (x) and without saponification (y). *ND=not detected. HO- high oryzanol, LO- low oryzanol

Table 2 shows the composition of unsaponifiable matters in RBOs. Tocopherols, tocotrienols, oryzanols, phytosterols and ferulic acid are typical compounds that can be found in unsaponfiable portion. In this study, α-tocopherol had been detected by fluorescence detector. Alpha -tocopherol is abundant in oil and it is the main type of tocopherol or vitamin E in oil. The three different types of oil extractions contained different concentrations of α-tocopherol. In this study Soxhlet RBOs contained significantly ($p<0.05$) higher amount of α-tocopherol compared to HO and LO which was 23.12±3.0 mg/g oil. In contrast, the study by Sarmento et al. (2006) found that the concentration of tocols in hexane extraction was lower compared to SFE due to higher temperature during solvent extraction and longer time of oil exposed to air. There was no significant difference between *α*- tocopherol in HO and LO. The concentration of *α*-tocopherol in HO and LO was 11.61±0.8 mg/g oil while LO 6.47±0.01 mg/g oil. The possibilities of higher amount of tocopherol in RBO might be due to the solubility of this compound in hexane. The concentration of tocotrienol in HO, LO and Soxhlet RBOs was 2.62±0.2 mg/g oil, 3.24±0.6 mg/g oil and 2.45±0.05 mg/g oil, respectively. Sarmento et al. (2006) found that SFE yielded a high amount of tocopherol and tocotrienol particularly at 200bars and 40 ºC.

In the three types of oils using different extraction method, the compound of oryzanol or gamma-oryzanol was detected using a diode array detector (DAD). In this study, there was a significant difference ($p<0.05$) of the oryzanol content of the different type of oil extractions. Table 2 shows HO extracted using SFE was significantly containing high amount of oryzanol as compared to LO and Soxhlet extraction. Concentration of oryzanol in HO was 7.84±0.04 mg/g oil while in LO and Soxhlet RBO was 6.17±0.02 mg/g oil and 4.81±0.11 mg/g oil. Xu and Godber (2000) found that SFE extraction yields four times more gamma- oryzanol in rice bran oil compared to solvent extraction. This is due to the effect of higher temperature in SFE system that alters the physical properties of rice bran matrix. The alteration of rice bran matrix makes it more penetrable by the extraction fluid of SFE (Xu and Godber (2000). Quantification of gamma-oryzanol in the oil with and without the saponification was done to compare the concentration of gamma-oryzanol in rice bran oil. Saponification process was an important process in most lipid extracted samples because it can remove interfering triglycerides and other hydrolysable materials. Besides, it also aids the release of lipids from a sample matrix. However, gamma- oryzanol that undergo the saponification process was not detected. This is

because bonds between ferulic acid and triterpene alcohol could be hydrolyzed under alkali condition (Xu and Godber 2000). In this study, 4 major isomers were detected by HPLC in RBOs samples (Fig. 2). These isomers were cycloartenyl ferulate, 24-methylene cycloartanyl ferulate, campesteryl ferulate and beta- sitosteryl ferulate. In this study, ferulic acid could not be detected by a detector in the HPLC assay. Ferulic acid is a part of oryzanol, appeared as a mixture of ferulic acid esters of phytosterols. To obtain the ferulic acid component oils needs to be hydrolyzed to break down the bond between ferulic ester of phytosterol.

3.1.2 Analysis of neutral lipid of rice bran oils

In this study, neutral lipid was separated by HPLC using a C18 Supelco column into monoacylglycerol (MAG), diacylglycerol (DAG), triacylglycerol (TAG) and free fatty acids (FFA) classes. Separation of components in NL using the HPLC was depending on the polarity of components. In this study MAG was eluted at an earlier retention time as this component more polar than TAG, while TAG as the least component that was eluted.

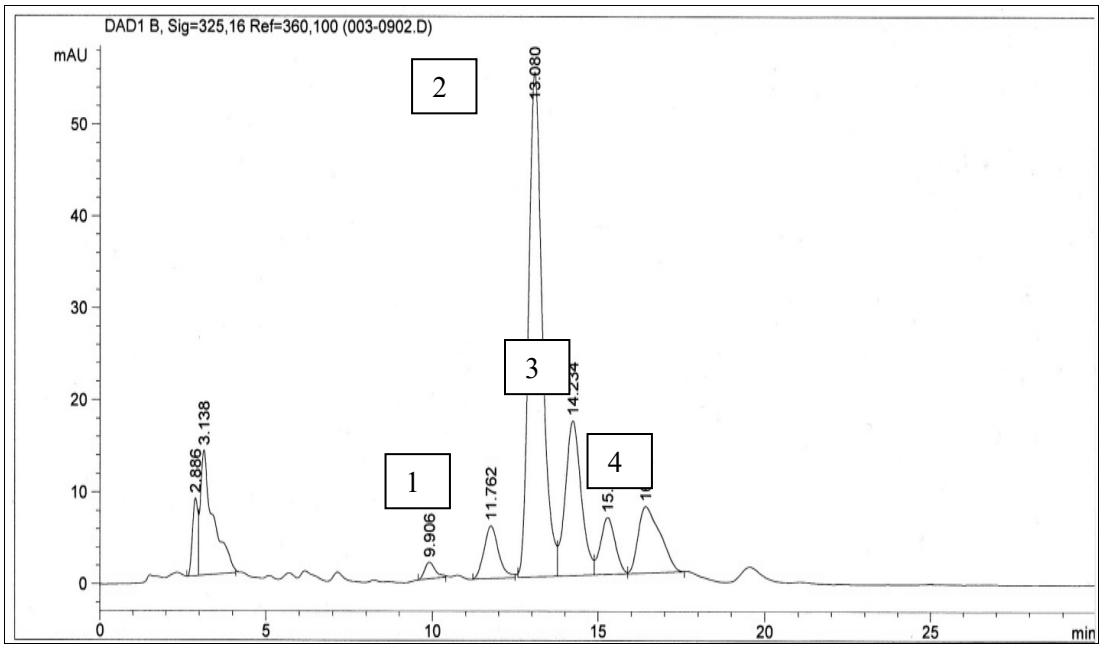

Figure 2. Ultraviolet detection of gamma-oryzanol in HO extracted by SFE. 1= cycloartenyl ferulate; 2= 24-methylene cycloartanyl ferulate; 3= campesteryl ferulate; 4=–β-sitosteryl ferulate and cycloartanyl ferulate.

Table 3. Components of Neutral Lipid in Different Extracted of Rice Bran Oil

Sample	MAG	DAG	TAG	FFA
High oryzanol SFE(mg/g)	32.05±3.0^{a}	14.05±0.3^{a}	137.52±0.6^{a}	39.22±4.8^{a}
Low oryzanol SFE(mg/g)	56.09±1.3^{b}	15.51±0.15^{b}	135.28±0.4^{ab}	46.52±4.4^{a}
Soxhlet(mg/g)	29.94±5.03^{a}	15.01±0.74^{b}	138.09±1.6^{b}	37.63±3.0^{a}

a-b: The different letters within the same column indicate a significant difference ($p < 0.05$). Abb: MAG-monoacylglycerol, DAG- diacylglycerol, TAG- triacylglycerol, FFA- free fatty acid

According to Table 3, MAG in LO (56.09±1.3 mg/g oil) was significantly ($p < 0.05$) higher than HO (32.05±3.0) and Soxhlet oil (29.94±5.03). Meanwhile, the amounts of MAG in both HO and Soxhlet extractions were not significantly different. DAG compound in the three different types of oil extractions contributed about 1-2%. This was slightly different with data reported by Orthoefer, (2005) who reported that DAG was about 2-4% (Orthoefer, 2005). In this study, the amount of DAG in HO and LO that have been extracted using SFE was 14.05±0.3 mg/g oil and 15.51±0.15 mg/g oil. Meanwhile, the amount of DAG in Soxhlet oil was slightly similar with LO which was 15.01±0.74 mg/g oil. Study by Marimuthu et al. (2010), found rice bran oil contain high amount of DAG which was 2.46%. DAG in vegetable oil is formed by oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) acids in a ratio of 7:3:1. Thus, at high temperature and pressure of the SFE extraction the compounds of DAG might be maximized. There was a significant difference ($p<0.05$) of TAG in Soxhlet and HO. TAG of Soxhlet RBO contributed 138.085±1.6 mg/g oil (13.81%) compared to HO and LO which were 137.52±0.6 mg/g (13.75%) and 135.28±0.4 mg/g (13.53%) of oil, respectively**.** However, the finding was in contrast with previous study where the amount of TAG of oil approximately more than 80% (Krishna et al. 2006). This is due to the solubility problems presented by solid glycerides in each mono- , di-, and tri-. It is difficult to find a solvent as mobile phase in which this component soluble and can be separated in reverse phase HPLC (Marimuthu et al. 2010). There was a high amount of FFA mainly oleic acid in LO compared to HO and Soxhlet RBO ($p>0.05$). Amount of FFA in LO was 46.52±4.4 mg/g oils compared to HO and Soxhlet oil, which was 39.22±4.8 mg/g oil and 37.63±3.0 mg/g oil, respectively.

3.1.3 Fatty acid composition in rice bran oil

The fatty acid methyl ester (FAME) analysis was quantified using Thermo Fisher Scientific Triple Quad GCMS (St. Waltham, MA, USA). The experiment was carried out in duplicate due to the limited sample and time constraint. The result shows that RBOs contain myristic acid (C14), palmitic acid (C16), heptadecanoic acid (C17), stearic acid (C18), oleic acid (C18:1), linoleic acid (C18:2), arachidic acid (C:20) and eicosenoic acid (C20:1). The amount of fatty acid in RBOs samples per one gram of oil is shown in Table 4.

Table 4. Fatty Acid Composition in Rice Ban Oil Samples

	Sample (g/g)		
Fatty acids composition	**High oryzanol SFE**	**Low oryzanol SFE**	**Soxhlet**
C14:0	0.028±0.01	0.027± 0.01	0.029± 0.02
C16:0	0.163±0.01	0.058± 0.06	0.030±0.01
C17:0	0.013±0.02	0.00± 0.06	0.025±0.01
C18:0	0.029±0.00	0.008±0.01	0.010±0.02
C18:1	0.316±0.01	0.059± 0.08	0.053± 0.01
C18:2	0.484±0.02	0.120± 0.04	0.095±0.01
C20:0	0.020± 0.00	-	-
C20:1	0.072± 0.02	-	-
Saturated fatty acid	0.252 ± 0.2	0.093 ± 0.1	0.094± 0.03
Monounsaturated fatty acid	0.388±0.01	0.059±0.08	0.053±0.01
Polyunsaturated fatty acid	0.484±0.02	0.120±0.04	0.095±0.01

These results were not consistent, particularly for LO and Soxhlet RBOs and might be due to an error during FAME preparation. During the FAME preparation, transmethylation reaction showed a precipitate in solution. This might be due to the high content of wax in RBO samples. Transmethylation is much better if dewaxed oil is used for fatty acid analysis. According to Table 4, eight fatty acids were detected in HO compare to LO and Soxhlet RBO. The highest amount of fatty acids in HO was oleic acid and linoleic acid, which was 0.316±0.01 g/g and 0.484±0.02 g/g oils. Kim et al. (2005), reported that SFE of RBO yielded more essential fatty acids (EFA) compared to solvent extraction. In addition, the amount of MUFA and PUFA in HO oils was higher compared to LO and Soxhlet RBO. Amount of MUFA and PUFA in HO was 0.388±0.011 g/g oil and 0.48±0.02 g/g oil.

4. Conclusions

Comparing supercritical fluid and Soxhlet extraction systems (SFE and SE) for concentrating bioactive components of rice bran oil. A distinctive concentrations of tocopherols, tocotrienols, and oryzanols were separated by the two methods. Oryzanol in RBO demonstrated a significant difference ($p < 0.05$) between diverse fractions of RBOs. Consequently, extraction by SFE impacted the sum, composition, and antioxidant of lipid in RBOs

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EFFECT OF LEMON (*CITRUS LIMON* L.) ADDITION TO *Pluchea indica* Less BEVERAGE

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ABSTRACT

This study was conducted with the aim of estimating quantitative changes in physicochemical, and sensory properties, antioxidant and antidiabetic activities of *Pluchea indica* Less leaves beverage caused lemon juice addition. The previous research has showed that the drink of 2 g dried pluchea leaves powder has the highest sensory acceptance but it owns the lowest antioxidant activity. The phytochemical contents of lemon juice expected can increase the antioxidant activity of this beverage. The lemon juice at various concentrations (0, 1, 2, 3, 4, and 5 % v/v) was added in 100 mL of hot water (~95ºC) extract for 5 min from dried Pluchea leaves powder in tea bag packaging. Parameters were tested physicochemical properties including turbidity, color, pH, total acid; antioxidant and antidiabetic activities and sensory properties comprising taste, color, and aroma. The results showed that the addition of lemon juice at various concentration can increased turbidity, lightness, total acid, total phenolic content, total ascorbic acid, total flavonoid, antioxidant activity and antidiabetic activity and decreased pH of beverages. The existence of phytochemical compounds of beverage from lemon juice and pluchea leaves gave to contribute the interaction of their constituents that were influenced to physicochemical and sensory properties, antioxidant and antidiabetic activities. This case study, it estimated that the organic acid content, especially citric acid and ascorbic acid from lemon juice could hydrolyze of glycoside bond or ester bond of phytochemical compounds in hot water extract of Pluchea leaves that could increase antioxidant and antidiabetic activities.

1.Introduction

Functional beverage usually is consumed by people to preserve body health. *Pluchea indica* Less, a herb plant including *Asteraceae* family, has been used as herbal drink (Srisook et al., 2012; Widyawati et al., 2016) because pluchea leaves water extract contains phytochemical compounds, such as flavonoid, saponin, tannin, phenolic, alkaloid, and cardiac glycoside (Widyawati et al., 2016; Widyawati et al., 2014). Andarwulan et al. (2010) also reported that pluchea leaves contain quercetin 5.21 mg/100g fresh weight and kaempherol 0.283 mg/100g fresh weight.

The previous research founded that 2g dried pluchea leaves powder in tea bag packaging brewed in 100 mL of hot water (~95ºC) results the highest sensory acceptance but the lowest antioxidant activity. The lemon addition of pluchea beverage was hoped to increase antioxidant activity. Zhou et al. (2015) reported that there are more than 170 antioxidants in citrus fruits, such as vitamins, mineral elements, phenolic compounds, terpenoids, and pectin. The vitamin C is a major vitamin in citrus fruit

that is a water-solubility substance. It can scavenge free radical, such as reactive oxygen species (ROS) and give off semi dehydroascorbic acid, clearing $^1O_2$ and reducing sulfur radicals. Se content of citrus fruit (0.5 μg/100 g) can be acted as antioxidant to destroy free radical cytoplasm and protect the tissues against oxidative damage. Phenolic compounds in citrus fruit, such as flavonoid, phenolic acid and coumarin are potential as antioxidant. Vandercook & Stephenson (1966) explained that phenolic compounds in lemon usually are formed aglycones and glycosides structures. There are some varieties of the coumarin compounds in lemon juice, such as 5-geranoxypsoralen, 8-geranoxypsoralen, 5-geranoxy-7-methoxycoumarin, 5,7-dimethoxy coumarin, oxpeucedanin hydrate, and byakangelicin. Tyagi et al. (2005) said that coumarins possess strong antioxidant activities because of their phenolic hydoxyl groups. Zhou et al. (2015) described that there are flavonoid compounds in citrus fruit having antioxidant activity, i.e. naringin, hesperidin and naringenin. The major flavanones compounds in lemon juice are hesperidin. This compound can scavenge DPPH free radical, inhibit Cu^{2+}-induced oxidation of low density lipoprotein (LDL) in vitro, promote pancreatic β cells regeneration, and prevent the oxidative stress on the embryos of diabetic pregnant rats. The content of hisperidin in lemon is 22 mg/100g.

There are free phenolic compounds from pluchea leaves and lemon juice in beverage caused interaction of hydroxyl groups so that influences the physicochemical and sensory beverage. The distance among the hydroxyl groups of phenolic compounds in beverage determines hydrogen bond formation so that establishes phenolic compounds solubility. Citric acid is major organic acid (1.44 g/oz) and ascorbic acid (Penniston et al. 2009) in lemon juice can hydrolyze glycoside bond or ester bond of phenolic compounds so that total aglycones components and solubility of them increase. Thereby the study was conducted to predict the effect of lemon juice addition to quantitative changes in physicochemical, and sensory properties, antioxidant and antidiabetic activities of *Pluchea indica* Less leaves beverage.

2. Materials and methods

2.1. Chemicals

Reagents used to analyze were *analytical grade,* including sodium acetate, chloroform, sulphuric acid, mercury chloride, potassium iodide, sodium hydroxide, folin ciocalteus phenol, cuppric sulphate, sodium nitrite, sodium dihydrogen phosphate, disodium hydrogen phosphate, methanol, eter, ethanol, ammonia solution, potassium ferric cyanide, trichloro acetic acid, acetic acid glacial, iodine, hydrochloride acid, n-amyl alcohol, magnesium powder, and ferric chloride were purchased by Merck Company (Darmstadt, Germany). Potassium sodium tartrate tetrahydrate, gallic acid, sodium carbonate, (+)-catechin, aluminium chloride, 2,2-diphenyl-1-picrylhydrazyl, alpha amylase enzyme, alpha glycosidase enzyme, amylum, and p-nitrophenyl-α-D-glucopyranoside were purchased from Sigma–Aldrich Company Ltd. (Gillingham, UK). Distillated water was supplied by PT Aqua Surabaya, Indonesia.

2.2. Materials

2.2.1. Plant Samples

Pluchea leaves were collected from a pluchea garden in manggrove area, Wonorejo, Rungkut, Surabaya, East Java, Indonesia. The plant was authenticated in the Herbarium of Biology and Food Industry Microbiology Laboratory at the Department of Food Technology, Agricultural Technology Faculty, the Widya Mandala Catholic University of Surabaya with voucher specimen no FTP-UKWMS-0001 for future reference (Widyawati et al. 2017). These leaves were sorted and graded based on age level and selected to get whole leaves. And then this leaves were used as samples. Lemon fruit were purchased from Hokky Supermarket in Surabaya, East Java, Indonesia with skin characteristic such as bright yellow color, smooth, and hard texture. Mineral water from commercial product was bought

from Bilka Supermarket in Surabaya, East Java, Indonesia. Tea bag was purchased from CV Peri Akas in Kwarasan, DI Yogjakarta, Indonesia.

2.2.2. Sampling

Pluchea leaves were harvested from 1-6 age level (Widyawati et al. 2014). The leaves were dried at room temperature around 7 days and ground to a fine powder (40 mesh) after they were washed and drained. The moisture content was determined to be 14.96 % (db). And then dried leaves powder was mixed before used.

2.2.3. Preparation of Pluchea Lemon Juice Beverage

Dried pluchea leaves powder was weighed 2 g in tea bag. And then it was extracted by 100 mL hot water (~95ºC) and mixed for 5 min. Lemon juice at various concentrations (0, 1, 2, 3, 4, and 5 % v/v) was added after sample temperature similar to ambient temperature around 15 min. Then this beverage was mixed and analyzed further.

2.2.4. Physicochemical Analysis

Turbidity of samples was analyzed based on Giwa et al. (2012). Turbidity is turbid condition or transparency reduction of liquid because there is a suspended particle in liquid. The quantity of beam absorbed is principled of turbidity measurement (Turbidity meter 966 IR, Orbeco Hellige, USA) (Omar et al. 2009). The higher of NTU (Nephelometric Turbidity Unit) is the bigger of turbid. The potential hydrogen is analyzed by AOAC (2005) method (pH meter Schott Lab 850, Germany). Principle analysis of pH is measurement of free hydrogen ion stated as acidity or alkalinity of samples. Total acid is measured by volumetric analysis (AOAC 2005). Principle analysis of total acid is neutralization reaction between hydrogen ion of acid and hydroxyl ion of base resulted water molecule. Sodium hydroxide 0.01 N called the titrant or titrator was prepared as a standard solution and phenolphthalein 1% (w/v) was used as a indicator. Color of samples was analyzed by Color Reader (Color Reader CR 20, Minolta, Japan) with using hunter system to determine L^*, a^*, and b^* values (McDaugall 2005). L* value is lightness having value between 0 (black) and 100 (white). a* value is redness showing mixed chromatic color between red (+a*) and green (-a*). b* value is yellowness having value between yellow (+b*) and blue (-b*).

2.2.5. Sensory Evaluation

Sensory evaluation was analyzed sensory based on hedonic preference test including aroma, taste, and color. Panelist number used was 80. Sensory assay used scoring test with 1-7 range. 1 score stated very dislike of samples and 7 showed very like of samples (Lawless 1999).

2.2.6. Phytochemical Composition

Phytochemical compounds identified including alkaloid, flavonoid, phenolic, triterpenoid, sterol, saponin, tannin, and cardiac glycoside (fehling test) were based on Harbone method (Harborne 1996). Identification of phytochemical compounds was showed with qualitative color of solution with color reader assay (Color Reader CR 20, Minolta, Japan) to determine color intensity.

2.2.7. Total Phenol Content Analysis

Total phenol content (TPC) was analyzed with folin ciocalteu's phenol reagent. The principle analysis is redox reaction between antioxidant compounds having aromatic ring and phosphomolybdate compound in folin ciocalteu's phenol reagent. The samples of beverage (100 mL) were added to 1 mL folin ciocalteu reagent 10%. After 5 min, 1 mL of 7.5% Na_2CO_3 was added, and then the mixture of samples was diluted by distillated water until 10 mL volume which was then left to stand for 30 min. Absorbance was read at 750 nm using a spectrophotometer (Spectrophotometer UV-Vis-1800, Shimadzu, Japan) and compared to gallic acid calibration curves. The content of total phenolics was expressed as mg gallic acid equivalent/L samples (mg GAE/L samples) (Singleton 1999).

2.2.8. Total Flavonoid Content Analysis

Total flavonoid content (TFC) was determined based on a stable acid complex compound formation of reaction between $AlCl_3$ and oxo group at C_4 ring and hydroxyl group at C_3 or C_5 ring of flavones and flavonol. Briefly, 200 µL of samples was added with 0.15 mL of

5% $NaNO_2$. After 5 min, 0.3 mL of 10% $AlCl_3$ was added. After another 5 min, 2 mL of 1 mol/L NaOH was added to the mixture. And then the samples were diluted by distilated water until 10 mL volume. Immediately, the absorbance of the mixture was determined by a spectrophotometer (Spectrophotometer UV-Vis-1800, Shimadzu, Japan) at 510 nm versus prepared water blank. Total flavonoids of samples were expressed as mg catechin equivalent/L samples (mg CE/L samples) (Al-Temimi & Choudhary 2013).

2.2.9. Ascorbic Acid Content Analysis

Ascorbic acid is a phytochemical compound that is acted as antioxidant (Taylor 1993) because this compound can donor electron so that it can prevent oxidation (Padayatty 2003). 250 µL of samples was diluted by distillated water until 10 mL volume and then the samples were mixed. Ascorbic acid content was analyzed by a spectrophotometer (Spectrophotometer UV Vis-1800, Shimadzu, Japan) at λ 265 nm based on Hassan et al. (1999) method.

2.2.10. DPPH Free Radical Scavenging Activity Analysis

The principle analysis is electron or hydrogen donating of antioxidant compounds to DPPH free radical colored purple to form DPPH-H non radical colored yellow. In this assay, 15 µL of samples with different concentrations of lemon juice addition was added with 1.5 mL of 60 µM methanolic-DPPH and added with methanol 1.5 mL. The mixture was shaken vigorously using vortex and left to stand for 30 min at room temperature in a dark room, and then samples were measured by a spectrophotometer (Spectrophotometer UV-Vis-1800, Shimadzu, Japan) at λ 517 nm with gallic acid compound as standard (Brand-Williams et al. 1995). The radical scavenging percentage using the following equation: Percentage (%) of DPPH free radical scavenging =

$$\frac{(\mathrm{Ab}-\mathrm{AS})}{(\mathrm{Ab})} \times 100\% \quad (1)$$

Where,

Ab that absorbance of the blank solution (DPPH–methanolic solution), AS that absorbance of samples.

2.2.11. Iron Reducing Power Analysis

This analysis is used to determine of the antioxidant capacity to reduce Fe^{3+} to Fe^{2+} (Chanda & Dave 2009). The pluchea-lemon beverages (50 µL) were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferric cyanide [$K_3Fe(CN)_6$] (2.5 mL, 1%). The mixture was incubated at 50°C for 20 min. A portion 2.5 mL of trichloro acetic acid (10%) was added to the mixture, which was then centrifuged at 1000 rpm for 10 min. The upper layer of 2.5 mL solution was mixed with 2.5 mL distilled water and 0.5 mL $FeCl_3$ 0.1%. The complex compound from ferri ferro cyanide colored Berlin blue was determined by a spectrophotometer (Spectrophotometer UV-Vis-1800, Shimadzu, Japan) at λ 700 nm with gallic acid compound as standard (Al-Temimi & Choudhary 2009).

2.2.12. In Vitro Inhibitory Alpha Amylase Assay

The analysis of in vitro inhibition alpha amylase was done by modified Odhav et al. (2010) method. All of pluchea-lemon beverages with various concentrations of lemon juice were taken 500 µL and added with 500 µL of amylum 1% (dissolving 1g of amylum in 100 mL of distilled water with boiling and stirring for 15 min). And then 500 µL of sodium acetate buffer at pH 5 was added and mixed. 250 µL of samples was mixed by 250 µL alpha amylase enzyme solution (0.1g of α-amylase 12.5 unit/mL in 50 mL of 0.2 M sodium acetate at pH 5. Furthermore, the mixture was added by 2 mL sodium hydroxide solution 1M and incubated at 37°C for 10 min. The absorbance was measured at 540 nm by a spectrophotometer (Spectrophotometer UV-Vis-1800, Shimadzu, Japan). Lemon juice addition at different concentrations (0, 1, 2, 3, 4, and 5 mg/mL) was performed in four replicates. Individual blank was performed by replacing enzyme with buffer. Control was performed by replacing sample with solvent. The inhibition percentage of α-amylase was assessed by the following formula:

$$\frac{(\mathrm{ACb}-\mathrm{ACa})-(\mathrm{AS}-\mathrm{Ab})}{(\mathrm{ACb}-\mathrm{ACa})} \times 100\% \quad (2)$$

Where,
ACb that absorbance of 100% enzyme activity (only solvent with enzyme), ACa that absorbance of 0% enzyme activity (only solvent without enzyme), As that absorbance of test sample with enzyme, Ab that absorbance of test sample without enzyme.

2.2.13. In Vitro Inhibitory Alpha Glycosidase Assay

The alpha glycosidase inhibitor assay was done by Mayur et al. (2010) with slight modification. 50 µL of samples was added with 50 µL 2mM P-nitrophenyl-α-D-glucopiranoside (PNP) (0.0150 g in 100 mL of 0.2 M sodium phosphate buffer (pH 7) used as a substrate to the mixture of 50 µL of α-glucosidase (0.0833 unit/mL). The reaction was conducted at 37℃ for 15 min and stopped by the addition of 1000 µL of 0.2 M Na_2CO_3. α-Glucosidase activity was assessed by measuring the release of p-nitrophenol from pNPG at 405 nm. Lemon juice addition at different concentrations (0, 1, 2, 3, 4, and 5 mg/mL) was performed in four replicates. Individual blank was performed by replacing enzyme with buffer. Control was performed by replacing sample with solvent. The inhibition percentage of α-glycosidase was assessed by the following formula:

$$\frac{(\mathrm{ACb} - \mathrm{ACa}) - (\mathrm{AS} - \mathrm{Ab})}{(\mathrm{ACb} - \mathrm{ACa})} \times 100\%$$

Where,
ACb that absorbance of 100% enzyme activity (only solvent with enzyme), ACa that absorbance of 0% enzyme activity (only solvent without enzyme), As that absorbance of test sample with enzyme, Ab that absorbance of test sample without enzyme.

2.2.14 .Statistical Analysis

The results were expressed as mean ± standard deviation of four replicates. ANOVA was used to execute the analysis of significant difference (α = 5%) with SAS (SAS/STAT version 17.0, SAS Institute Inc., Cary, NC, USA) if that test is significantly different followed by Duncan's Multiple Range Test at α = 5%).

3. Results and discussion

3.1. Physicochemical properties

The change of physicochemical properties from pluchea leaves beverage with lemon juice addition at various concentrations was showed at Figure 1 and 2. The appearance of pluchea-lemon beverage after 15 min was extracted by hot water at ~95ºC for 5 min was showed at Figure 3.

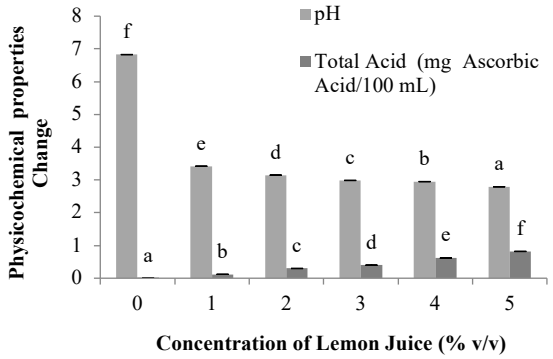

Figure 1. The effect of lemon juice addition at various concentrations of pH and total acid changes from pluchea-lemon beverage

Lemon juice addition caused significant different of increased turbidity, total acid and lightness but pH value of beverages showed significantly decreased ($p < 0.05$).

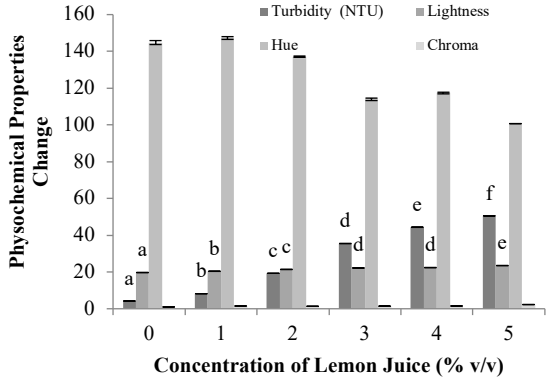

Figure 2. The effect of lemon juice addition at various concentrations of turbidity, hue, lightness and chroma values from pluchea-lemon beverage

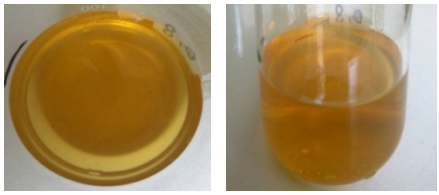

Figure 3. The appearance of pluchea-lemon beverage after 15 min was extracted by hot water at ~95ºC for 5 min

The possible of explanation for the turbidity change in pluchea leaves beverage was caused by total soluble solid (TSS) increasing. Lemon (*Citrus limon* cv Eureka) has 8.97 % TSS (Al-Juhaimi & Ghafoor 2013), it is contributed by phytochemical compounds, such as mineral, vitamin, carbohydrate, protein, copper, calcium, vitamin B9, B1, B3, B6, phenolic (Fernandez-Lopez et al. 2005; Jayaprakasha et al. 2008), flavonoids, coumarins, limonoids, carotenoids, pectins and other components (Zhou 2015). The solubility of phytochemical constituents in beverages determined turbidity value. Generally, phenolic compounds in lemon juice have free and bond structures (Vandercook & Stephenson 1966), it is similar as the phenolic compounds of water extract of pluchea leaves powder so that the addition of lemon juice in this beverage caused interaction of hydroxyl groups among phenolic compounds to form insoluble macromolecules. Siebert et al. (1996) said that tannin acid, catechin and gelatin can cause haze increasing in wine, beer and fruit juices. The precipitating ability of polyphenols increases as the number of o-diphenol groups in the molecule increases. Tannic acid or proanthocyanidin dimers or trimers is more effective to make haze in beverages than catechin having one o-diphenol group and one m-diphenol group.

The some studies have suggested that lemon contains potasium, phosphor, magnesium, and E, kolin, ascorbic acid, flavonoid, B2, and B5 (Molina et al. 2010), vitamin A and the phytochemical compounds. Pluchea leaves beverage has brown yellowish color, because pluchea leaves contain tannin (Widyawati et al. 2016). Lemon juice addition of samples can increase lightness value of this beverage. The lightness change of samples was related with pigment color from lemon fruit, chlorophyll and its derivate give green to yellow color. However the beverage lightness value increasing didn't contribute to hue and chroma values change. It means the interaction of molecule structures in phenolic compounds from pluchea leaves and lemon is not influenced beverage color. Although Tapas et al. (2008) said that the different molecule structures of phenolic compounds are responsible to color of beverages.

pH and total acidity of lemon fruit juice are 2.81 and 6.49 ± 0.01 g/L, respectively (Al-Musharfi et al. 2015). The major organic acids in lemon are citric acid (1.44 g/oz) and ascorbic acid (Penniston et al. 2009), and then the dominan organic compounds in pluchea leaves is chlorogenic acid and caffeic acid (Apriady 2010). The soluble organic compounds in pluchea-lemon beverage determined total acid and pH, were depended on the added lemon juice concentration, the maturity of lemon fruit determines total soluble solid and acid content.

3.2. Sensory properties

Sensory properties of pluchea-lemon beverage were showed at Figure 4. From the evaluation of panelist based on hedonic preference test was indicated significant different ($p < 0.05$) in the color, taste and aroma of beverages. Data showed that aroma acceptance increased corresponding to the progress of lemon juice concentration. It must be noted that as essential oil in pluchea leaves and lemon fruit give contribution to the aroma of the drink. Traithip (2005) informed that pluchea leaves are comprised volatile compounds, such as boehmeryl acetate, HOP-17 (21)-ene 3β-acetate, linaloyl glucoside, linaloyl apiocyl glucoside, plucheoside C, cuauhtermone, 3-(2'-3'-diacetoxy-2'methyl-butyril), plucheol A, plucheol B, plucheoside A, plucheoside B, plucheoside E, pterocarptriol, sequisterpene, monoterpene, and triterpene. Widyawati et al. (2013) also found that essential oil in pluchea leaves is composed alcohol (6.16%), aldehide (1.79%), aliphatic unsaturated hydrocarbon

(1.35%), ester (0.08%), keton (3.49%), eter and sulphoxide (0.06%), aromatic hydrocarbon (2,05%), heterocyclic hydrocarbon (0.05%). Hui (2010) said that monoterpene (C10) and sequisterpene (C15) of lemon fruit give aroma specific. Volatile compounds from pluchea leaves and lemon fruit contributed of the drink aroma.

The taste and color acceptances of samples were significant increased at lemon juice concentration addition until 3 %. The declining acceptance of taste was caused by sour taste contributed by ascorbic acid and citric acid from lemon fruit, whereas the color acceptance was influenced by yellow color intensity increasing from samples because of soluble carotenoid pigment from lemon juice. Jokic et al. (2014) informed

Table 1. Phytochemical analysis of pluchea leaves beverages with lemon juice addition at various concentrations

Phytochemical Compounds		Concentration of Lemon Juice (% v/v)					
		0	1	2	3	4	5
Alkaloids	**Meyer**	+1	+2	+2	+3	+4	+5
	Wagner	+1	+1	+2	+2	+2	+3
Flavonoids		+2	+2	+2	+3	+3	+3
Polyphenols		+2	+2	+2	+3	+4	+5
Tannins		+2	+2	+3	+4	+4	+4
Saponins		+2	+3	+3	+2	+2	+1
Cardiac Glycosides		+	+	+2	+4	+4	+5
Triterpenoids		-	-	-	-	-	-
Sterols		-	-	-	-	-	-

Note : + Indicates : presence, - Indicates : absence

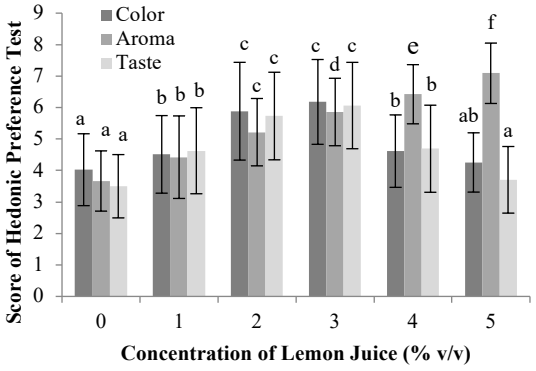

Figure 4. The effect of lemon juice addition at various concentrations of sensory properties from pluchea-lemon beverage

that phenolic compounds represent an important component of fruits and vegetables because they are significantly contributed to the taste, color and nutritional value of fruits and vegetables. Agbo et al. (2015) said that ascorbic acid is bioactive compounds in lemon fruit that is contributed to body health. The present of ascorbic acid in lemon fruit can influence sensory acceptance of panelist. Zhou et al. (2015) also described that vitamin C is soluble compounds in water contributed to taste of samples.

3.3. Phytochemical composition

Qualitative phytochemical analysis revealed the presence of tannins, flavonoids, polyphenols, alkaloids, saponins, and cardiac glycosides in pluchea-lemon beverage (Table 1). The lemon juice presence in this beverage increased detected phytochemical compounds quantity that was showed by color intensity of samples. It was proved that lemon fruit contains the phytochemical compounds mentioned. The phytochemical compound contents of the lemon juice are also observed by Mathew et al. (2012).

Whereas saponin detection had the different pattern, it was caused that saponin was absent in lemon juice. However Okwu (2008) informed that *Citrus limonum* has saponin content around 0.42 ± 0.01 mg/100g. The pattern change of saponin detection at phytochemical compounds identified was estimated by interaction among phytochemical compounds in pluchea leaves and lemon juice. The previous studies have informed that lemon fruit is composed with ascorbic acid, flavonoids, polyphenols, and pectins (Fernandez-Lopez et al. 2005; Jayaprakasha et al. 2008, Zhou et al. 2015), organic acids and essential oils (limonene, α-terpinene, α-pinene, β-pinene, citric acid, and caumarin (Molina et al. 2010), simple carbohydrate (glucose, fructose and sucrose) (Yekeler et al. 2013). Liu et al. (2004) said that saponins consist of a polycyclic aglycones attached to one or more sugar side chains. The aglycone part, which is also called sapogenin, is either steroid (C27) or a triterpene (C30). Saponin can be detected in samples based on the capability of saponin to make stable foam. The increasing of lemon juice concentration addition was estimated to cause glycoside bond of saponin hydrolyzed because of the interaction between organic acid compounds from lemon juice and glycoside bond from saponin. Whereas the lemon juice addition gave contribution of increasing from other phytochemical constituents

3.4. Total phenolic content, total flavonoid content and total ascorbic acid

The results of the qualitative phytochemical assay of the samples had the same pattern as the results of total phenolics (TPC), ascorbic acid (AAC) and total flavonoids (TFC) contents that were showed in Figure 5, 6 and 7.

It was observed that the lemon juice concentration increasing of the compared samples increased in the level of TPC, AAC, and TFC. Statistical analysis (Anova, p <0.5%) showed that there were significant different in the level of TPC, AAC, and TFC. It means that the bioactive compounds of lemon juice gave contribution to the TPC, TFC and AAC increasing in samples. The TPC, TFC, and AAC of samples were ranged from 225.42 ± 13.50 to 398.85 ± 13.09 mg GAE/L, 150.01 ± 3.87 to 214.98 ± 2.75 mg CE/L, and 18.40 ± 0.49 to 30.77 ± 0.71 mg AAE/L, respectively.

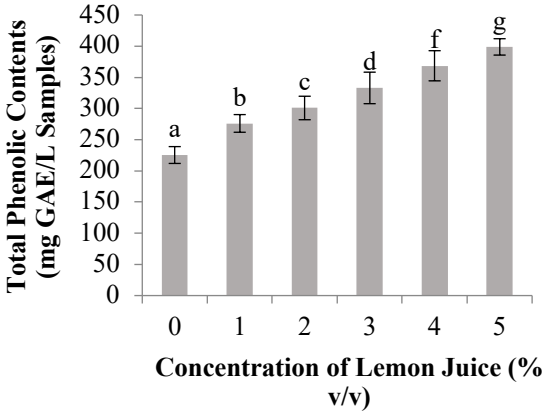

Figure 5. The effect of lemon juice addition at various concentrations of total phenols content (TPC) from pluchea-lemon beverage

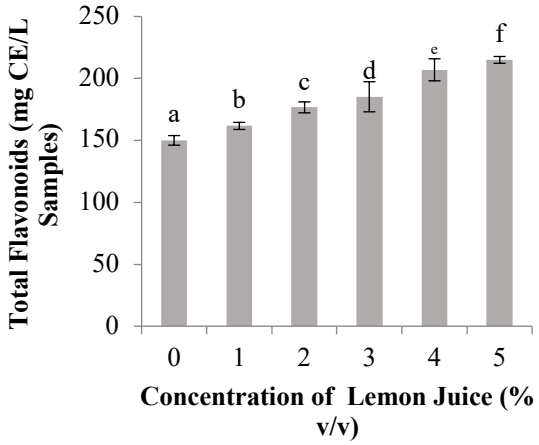

Figure 6. The effect of lemon juice addition at various concentrations of total flavonoids contents (TFC) from pluchea-lemon beverage

Phenolic compounds, including tannins, flavonoid, phenolic acids, qoumarine, quinine and other compounds, are bioactive compounds that are rich found in fruit juices (Bansode & Chavan 2012; Firdrianny et al. 2014; Lee et al. 2014). Zhou et al. (2015) also explained that citrus fruit phenolic compounds that have antioxidant activity such as flavonoids, phenolic acids and coumarins. The phenolic compounds are one of the most important groups of

secondary metabolites present in plants that are characterized by the possession of at least one aromatic ring carrying one or more hydroxyl groups (Rebaya et al. 2014). Phenolic compounds in fruits and vegetables usually form free or bond structures (Lim & Loh 2016).

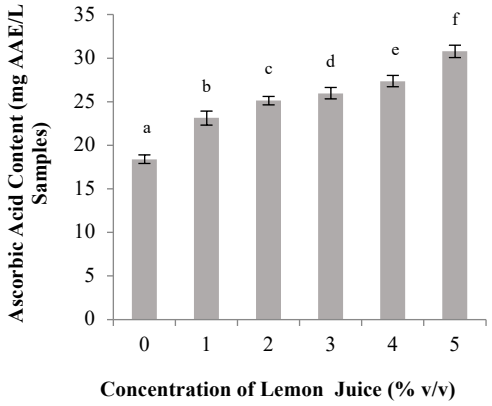

Figure 7. The effect of lemon juice addition at various concentrations of ascorbic acid content from pluchea-lemon beverage

Flavonoids are one class of phenolic compounds that are also known as vitamin P. These metabolites are mostly used in plants to produce yellow and other pigments which play an important role in the colors of plants (Calabro et al. 2004). The TPC of lemon fruit juice is 0.569 ± 0.031 mg GAE/mL (Porras et al. 2015). Flavanone, flavone and flavonol are a flavonoid compound group in lemon fruit (Mouly et al. 1994). Major flavonoid in lemon is hesperidin, narirutin, naringin and eryocitrin (Schieber et al. 2001; Andarwulan et al. 2010; Zhou et al. 2015), quercetin, tangeritin, and rutin (Yekeler et al. 2013). Phenolic and flavonoid in pluchea are quercetin, kaempherol, myricetin, luteolin, apigenin (Apriady 2010), caffeic acid and chlorogenic acid (Hajimahmoodi et al. 2012), Most of the fresh juices contain varying amount of water soluble vitamin C (ascorbic acid) which is the main nutritional component of these juices. The ascorbic acid content of lemon fruit juice is 0.616 ± 0.042 g/100 mL (Porras et al. 2015).

3.5. DPPH radical scavenging activity

The antioxidant activity was evaluated by two different spectrophotometric methods including DPPH and FRAP. The results were showed at Figure 8 and 9. It was demonstrated that the antioxidant activities of pluchea drink showed an increasing trend from low concentration to high concentration of lemon juice addition.

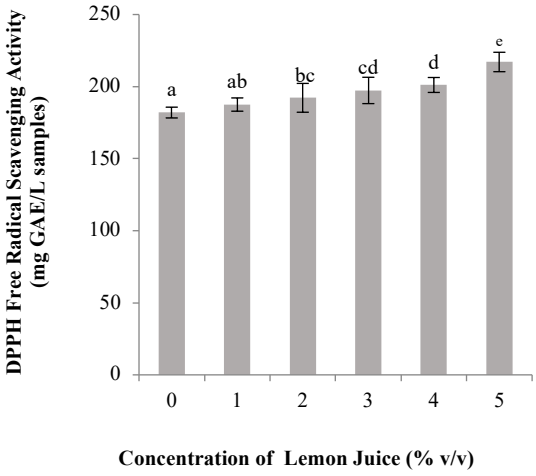

Figure 8. The effect of lemon juice addition at various concentrations of DPPH free radical scavenging activity from pluchea-lemon beverage

The scavenging activities for DPPH radical of samples was ranged from 181.98±3.76 to 217.08 ± 6.74 mg GAE/L samples. The lemon juice addition from 0 to 3 % (v/v) was obtained no significantly difference data, but this effect showed significant different at higher concentration addition ($p<0.5\%$). This antioxidant capacity assay involves hydrogen atom transfer, DPPH free radical can receive hydrogen atom to form DPPH-H that is observed with color change from purple to yellow (Chlopicka et al. 2012). Zhou et al. (2015) informed that vitamin C, A, and E, Se mineral, flavonoid, especially narigenin, naringin and hesperidin, phenolic acid, and coumarins, limonoids and pectins in citrus fruit are capable to scavenge free radical, such as reactive oxygen spesies (ROS) and peroxyl radical.

In this case study, steric accessibility is a major determinant of the analytical reaction. The small molecules of bioactive compounds are easier to react with DPPH free radical than the big molecules, because the small molecules have good access to reach the radical site (Stankovic 2011). This antioxidant activity was correlated with TPC, TFC and AAC.

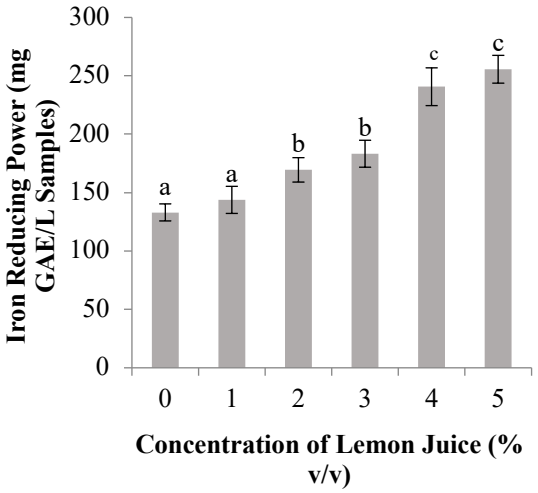

Figure 9. The effect of lemon juice addition at various concentrations of iron reducing power from pluchea-lemon beverage

3.6. Ferric reducing antioxidant power

The FRAP is antioxidant assay the corresponding concentrations of electron donating antioxidants and the compounds that act by radical quenching, i.e. thiol antioxidants (such as gluthathione) and carotenoids (Chlopicka et al. 2012). The ferric reducing power of samples was ranged from 133.03±7.30 to 255.64±11.89 mg GAE/L samples. The statistical data showed that the lemon juice addition at various concentrations of samples was significant different ($p<0.5\%$).

The antioxidant activity was similar to trend observed in TPC, TFC, and AAC of samples. Agbo *et al.* (2015) said that the high phenolic content of samples indicates high antioxidant capacity because the phenolics react with active oxygen radicals such as hydroxyl radical, superoxide anon radical, and lipid peroxy radical. There are correlation among antioxidant activity, TPC, TFC, and AAC. Stankovic et al. (2011) said that there are a high linear correlation between the values of phenol concentration and antioxidant activity. Phenol compounds can be acted as antioxidant due to their hydroxyl groups. Structure and substitution pattern of hydroxyl groups of phenolic compounds determine the antioxidant activity. Chlopicka *et al.* (2012) informed that among polyphenols the greatest antioxidant efficacies have antioxidant action, such as quercetin, tannic acid, caffeic acid, and gallic acid, while catechin and resveratrol have the lowest ones. The most effective antioxidants scavenging DPPH are gallic acid, tannin acid, ascorbic acid, and quercetin. Jablonska-Rys et al. (2009) explained that ascorbic acid is lower the antioxidant activity than the phenolic compounds. Therefore the antioxidant activity of pluchea drink was contributed by all of phytochemical compounds of lemon juice and pluchea leaves. The lemon juice addition containing phytochemical compounds caused the increasing of the antioxidant activity.

3.7. In vitro inhibitory alpha amylase activity

Previous research has showed that herbal plants can use to treat diabetes, as their principal bioactive components showed good anti-diabetic and anti-oxidant properties (Keerthana et al. 2013). The effect of lemon juice addition at pluchea-lemon beverage was showed at Figure 10. The alpha amylase inhibitory activity of samples was ranged from 55.30±2.90 % to 84.85±2.47%. The statistical data showed that the lemon juice addition at various concentrations of samples was significant different ($p<0.5\%$). There was a positive relationship between antioxidant activity and alpha amylase inhibitory activity. The activity was influenced by TPC, TFC and AAC. The potency of samples inhibited alpha amylase activity is determined by the presence of potential inhibitors such as tannins, phenols, flavonoids, saponins, steroids, alkaloids, terpenoids (Myung-Hee et al. 2010; Nanumala et al. 2015).

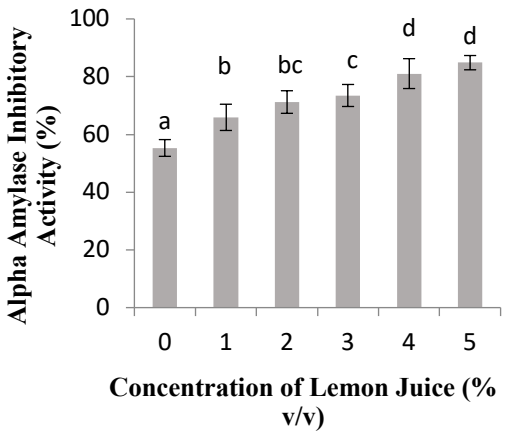

Figure 10. The effect of lemon juice addition at various concentrations of in vitro alpha amylase inhibitory activity from pluchea-lemon beverage

These alpha amylase inhibitors are also called as starch blockers since it prevents or slows the absorption of starch in to the body mainly by blocking the hydrolysis of 1,4-glycosidic linkages of starch and other oligosaccharides into maltose, maltriose and other simple sugars (Dineshkumar et al. 2010). The polyphenols and flavonoids have capability to bind with a active site of alpha amylase enzyme so that they can inhibit its activity. The ascorbic acid and citric acid existence in lemon juice are contributed as hydrolyzed agent to cleave a glycoside bond or ester bond in polyphenol compounds increasing a free polyphenols quantity. The free polyphenols have ability to bind with proteins (Fifa et al. 2013). Lim & Loh (2016) underlined that the free soluble phenolics had slightly higher inhibitory α-amylase activity than the bound phenolics. McCue *et al*. (2004) suggested that the effect of the free soluble phenolics to the five sets of disulphide bridges located on the outer surface of α-amylase can reduce of these cysteine residues so that causes inhibition by modifying in the structure of the enzyme.

3.8. In vitro inhibitory alpha glycosidase activity

The α-glucosidase enzyme is one of the key enzymes involved in dietary carbohydrate digestion in human. It hydrolyzes the carbohydrate, releasing glucose and cause the raised postprandial blood glucose level (Lee et al. 2014). In this study, the α-glucosidase inhibitory activity of pluchea-lemon beverage at various concentration of lemon juice was showed at Figure 11.

The alpha glycosidase inhibitory activity of samples was ranged from 67.86 ± 4.12 % to 89.29 ± 7.14%. The statistical data showed that the lemon juice addition at 0-2% (v/v) concentrations of samples was significant different with 3-5 % (v/v) concentrations ($p<0.5\%$). However there was trend that lemon juice addition increased alpha glycosidase inhibitory activity. There was a positive relationship among TPC, TFC, AAC, antioxidant activity and alpha glycosidase inhibitory activity.

This antidiabetic activity is contributed by coumarin compounds, such as 5-geranoxypsoralen, 8-geranoxypsoralen, 5-geranoxy-7-methoxycoumarin, 5,7-dimethoxy coumarin, oxpeucedanin hydrate, and byakangelicin in lemon juice (Vandercook & Stephenson 1966). Zhou et al. (2015) also explained that coumarins possess strong antioxidant activity because of their phenolic hydroxyl groups. The potency of alpha glycosidase inhibitory activity by caumarin compounds was ranged of IC_{50} values of 65.29-172.10 μM (Ali et al. 2016).

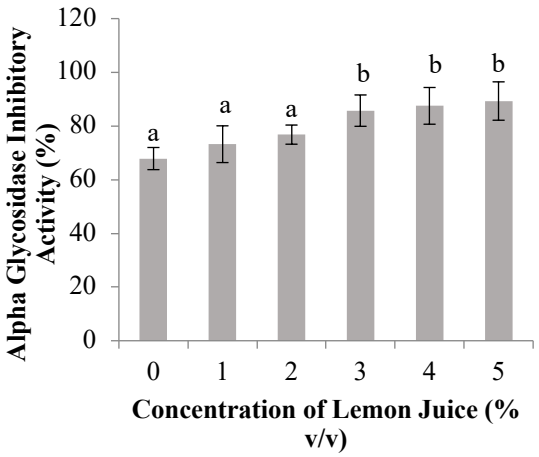

Figure 11. The effect of lemon juice addition at various concentrations of in vitro alpha glycosidase inhibitory activity from pluchea-lemon beverage

Zhao et al. (2015) also clarified that the coumarin compounds are against α-glucosidase activity with non competitive inhibition mode. The interaction between the coumarin compound and α-glucosidase was a spontaneous process that was driven mainly by hydrophobic force. Astriningtyas et al. (2014) said that phytochemical compounds of pluchea leaves also are contributed to alpha glycosidase inhibitory activity, such as 3,5-di-O-caffeoylquinic acid, 4,5-di-O-caffeoylquinic acid mehyl ester, 3,4,5-tri-O-caffeoylquinic acid methyl ester, 3,4,5-tri-O-caffeoylquinic acid and 1,3,4,5-tetra-O-caffeoylquinic acid.

Lim & Loh (2016) explained that the bound phenolic compounds have slightly higher inhibitory effect in α-glucosidase than the free phenolic compounds. The inhibitory mechanism of alpha glycosidase activity is none disulphide bridges especially not on the surface of the molecule (possible site for interaction with antioxidants) on the structure of Baker's yeast α-glucosidase but the inhibition is attributed through other mechanism. This our study, the lemon juice addition could cause a glycoside bond or ester bond of phenolic compounds cleavage so that the total free phenolic compounds in beverage increased. Therefore it was predicted that the presence of some non-phenolic phytochemicals was acted as enzyme inhibitors, exhibiting an additive or synergistic effect with the present of phenolics in the sample.

4. Conclusions

The lemon juice addition at various concentrations was influenced physicochemical, antioxidant, antidiabetic and sensory properties of pluchea-lemon beverage. The type and number of phytochemical compounds and interaction among them were contributed to physicochemical, antioxidant, antidiabetic and sensory properties of samples.

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POTENTIAL USE OF OLIVE OIL MILL WASTEWATER TO CONTROL PLANT PATHOGENS AND POST HARVEST DISEASES

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ABSTRACT

The antifungal activity of olive oil mill wastewater (olive OMW) was investigated. The effect of sterilized, filtered and non-sterilized olive OMW was tested *in vitro* a) on mycelium growth of *Pythium* spp., *V. dahliae, S. sclerotiorum* and *F. oxysporum* f.sp. *lycopersici* on PDA medium, b) on sporulation of *Penicillium* species and *Botrytis cinerea* on infected with the pathogen fruits (mandarin and red pepper) and c) on tomato plants (seedlings) infected with the fungus *V. dahliae* or *S. sclerotiorum* . The *in vitro* results shows that the filter sterilized olive OMW inhibits the growth of all tested fungi mycelium. Olive OMW decreased fungi spores (conidia) number on infected fruits and acted positively on tomato plant growth.

1.Introduction

During olive oil extraction a large amount of solid and aqueous residues as olive oil mills wastewaters (olive OMWs) produced annually worldwide where the majority of it being produced in the Mediterranean basin. The uncontrolled disposal of olive OMW is becoming a serious environmental problem due to its high content of phenolic compounds: tannins and flavonoids (Gonzales *et al*. 1999; Hamdi, 1992). Some of these phenols are responsible for several biological effects, including antibiosis (Rodrvguez *et al.* 1988) and phytotoxicity (Capasso *et al*. 1992). They also appear to be involved in the defense of plants against invading pathogens, including bacteria, fungi and viruses (Marsilio *et al.* 2001). The use of olive OMW for plant and harvested fruit protection against microorganism could be a solution for residues management and nature protection. The main objective on this study was to examine the antifungal activity of olive OMW against plant pathogens and plant post-harvest diseases.

2.Material and methods

2.1.Effect of olive OMW on the mycelium growth of fungus

The antifungal effect of olive OMW against plant pathogens (*Fusarium oxysporum* f.sp. *lycopersici, Pythium* spp., *Sclerotinia sclerotiorum* and *Verticillium dahliae*) were tested *in vitro*. Tested were made on PDA (Potato Dextrose Agar; DIFCO). Treatments were PDA plates with a) olive OMW added into the medium and autoclaved and b) a drop of filter sterilized olive OMW (using a syringe filler 0.2 μm) added onto the agar surface. In the first treatment a 25ml of olive OMW were added into 1l agar and further sterilized by autoclaving (120 ^{o}C for 20 min). In the second treatment a drop (50 μl) of sterilized filtered olive OMW was added onto the centre of each plate. Fifteen agar plates per treatment, were inoculated with a mycelium plug (5 mm in diameter) of the above fungi (depended of the treatment) taken from the periphery of 7 days old fungal colonies. Mycelia plugs were placed onto the centre of each plate or next to the olive OMW drops. Equal plate

numbers per fungus treatment were used as control (without olive OMW). Plates were incubated at 21ºC for six days and fungi mycelium growth was recorded.

2.2.Antimicrobial activity of olive OMW on fruits treated with pathogens

Two common species of *Penicillium* (*P. italicum and P. digitatum*), isolated from mandarin fruits and *Botrytis cinerea* isolated from red horn (sweet) peppers were used for this experiment. Spores suspension was prepared by collecting spores of above *Penicillium* species, from 8 days old cultures. Three agar plates per fungus culture were used to collect spores. Spores were collected in 1l Erlenmeyer flask which contained distilled water by washing the agar surface with 3ml distilled water and filtered that solution throw sterilized muslin. In each flask spores suspension was adjusted at 10^6 spores/ml. A 50ml of olive OMW were added in each flask. Mandarin fruits were surface sterilized and soaked for 3 min in 1l beakers contained 500 ml of the above spore and olive OMW solution. After that time fruits removed from the flasks, dried for 10 min in a laminar flow unit and incubated at 21ºC for 12 days. Olive OMW was passed through Whatman filter paper No 2 before added to each beaker. The same procedure was followed for red horn peppers inoculated with *B. cinerea.* After the incubation time, the spore number of each mandarin fruits or peppers were count by scraped each treated fruit surface into 1l beaker contained 500ml distill water. The spore number per treatment and per beaker was counted in optical microscope using a hematocytometer. The experiment had fourteen replicates per treatment and two treatments; infected with spores and olive OMW mandarin fruits and infected with spores and olive OMW red peppers. Equal numbers of mandarin fruits and red peppers soaked only in olive OMW and only in fungus spore suspension were used as control.

2.3.Effect of olive OMW on tomato plants infected with Fusarium oxysporum f.sp. lycopersici

In this experiment, two tomato varieties were used, cv. Roma and cv. Marmande. Plant pathogens used were *V. dahliae* and *S. sclerotiorum,* both isolated from tomato plants. For each variety, 42 tomato seedlings were incubated with spores (10^6 conidia/ml) of *V. dahliae* and 42 seedlings with *S. sclerotiorum* with mycelial suspension collected from 10 PDA petri dishes. In *V. dahliae* treatment from the 42 plants 21 of them were incubated in *V. dahliae* conidia suspension for 10 min and 21 were incubated in the conidial suspension treated with olive OMW (5 ml/100 ml in total solution). In *S. sclerotiorum* treatment, 21 tomato plants were incubated in *S. sclerotiorum* mycelial suspension for 30 min and 21 were incubated in mycelial suspension treated with olive OMW (5 ml/100 ml in total solution). Olive OMW was passed through Whatman filter paper No 2. All treated plants were planed into 250ml pots and kept in a glasshouse. Plants were harvested 45 days after planting and stem height were measured. Tomato plants (21plants/variety) were used as control.

2.4.Statistical analysis

Data were analyzed using the Minitab statistical package. Analysis of variance was used to assess treatments effect.

3.Results and discussion

3.1.Effect of olive OMW on the mycelium growth of fungus

There was a statistically significant difference between filtered olive OMW and control (untreated PDA and sterilized with olive OMW PDA), (P<0.001). The filtered olive OMW inhibits the growth of all tested fungi mycelium (Fig. 1). Sterilized olive OMW had similar effect on the mycelia growth of *Pythium* spp., *V. dahliae, S. sclerotiorum* and *F. oxysporum* f.sp. *lycopersici* with the untreated control. However, sterilised olive OMW seems to have some positive effect on the mycelium growth of all tested fungi (Fig 1).

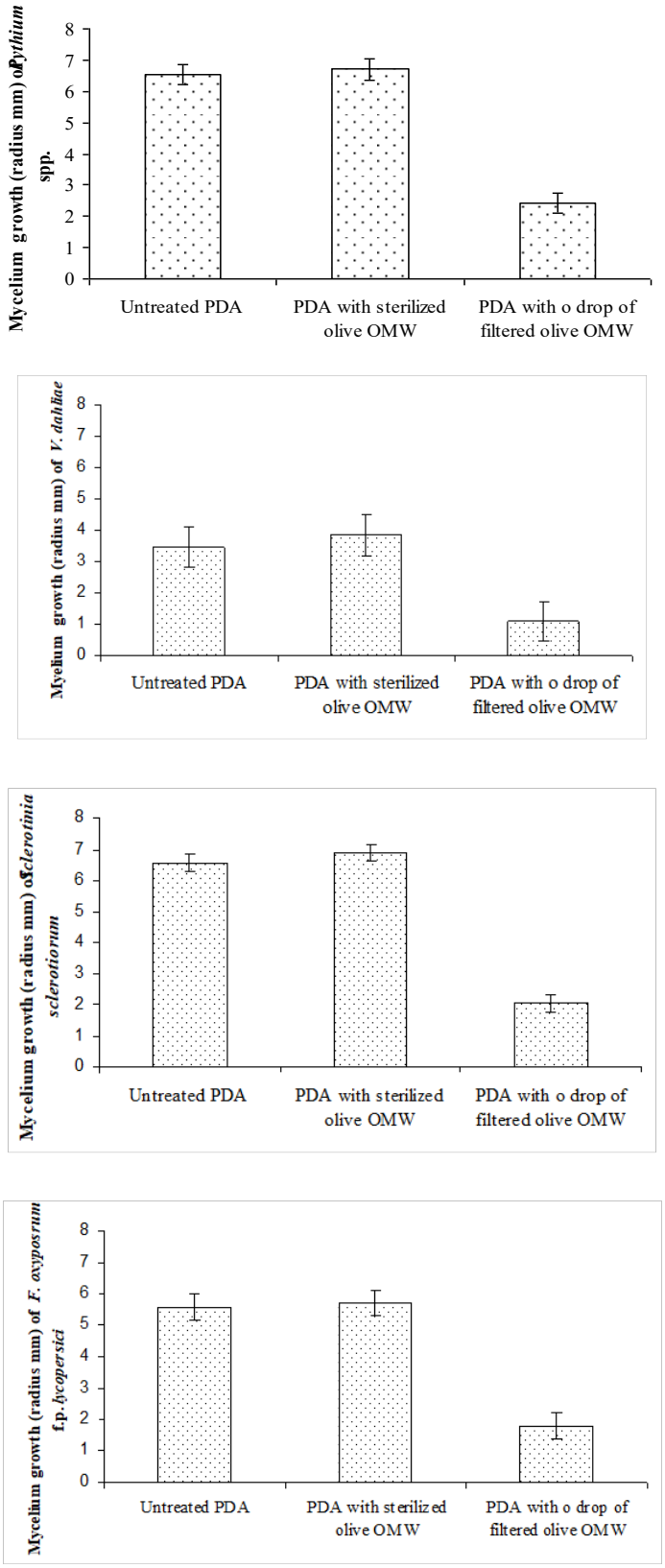

Figure 1. Effect of sterilized and filtered olive oil mill wastewater (olive OMW) on the mycelium growth of *Pythium* spp., *V. dahliae, S. sclerotiorum* and *F. oxysporum* f.sp. *lycopersici*.

3.2.Antimicrobial activity of olive OMW on fruits treated with pathogens

The olive OMW reduced the number of *Penicillium* spp. (P<0.001) and *B. cinerea* (*P*<0.001) spores from mandarin fruits and red peppers respectively. On mandarin fruits the average spore's number was $4,7x10^6$ for mandarin fruits infected only with *Penicillium* species and $0,9x10^2$ for mandarin fruits infected with *Penicillium* species and treated with olive OMW (Fig. 2). On red horn (sweet) peppers the average spore's number was $4.6x10^5$ for peppers infected only with *B. cinerea* and $2,2x10^2$ peppers infected with *B. cinerea* and treated with olive OMW.

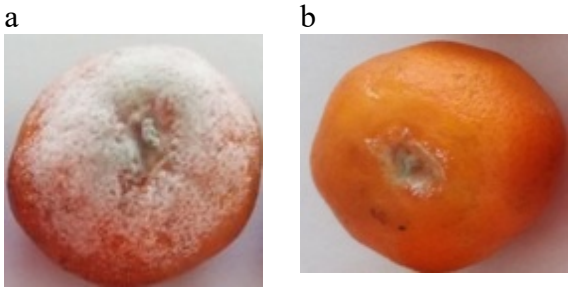

Figure 2. Mandarin fruits infected with *Penicillium* species only (a), with *Penicillium* species and treated with olive OMW (b).

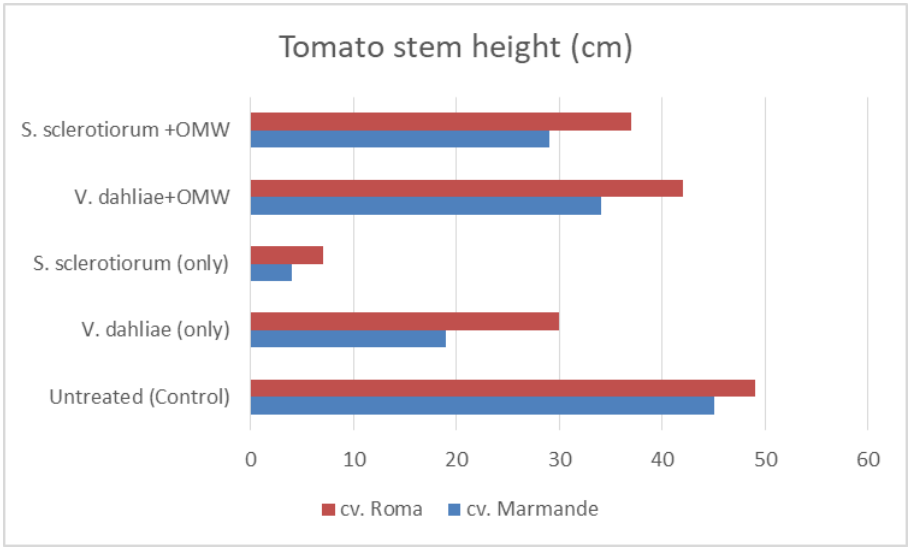

Figure 3. Effect of olive OMW on tomato stem height infected with *V. dahliae* spores or with *S. sclerotiorum* mycelia suspension.

3.3.Effect of olive OMW on tomato plants infected with *Fusarium oxysporum* f.sp. *lycopersici*

There was a statistically significant difference (*P*<0.001) between untreated (Control) and treated plants with *V. dahliae* spores or *S. sclerotiorum* mycelia suspension on the height of tomato stem (Fig. 3). Olive OMW produced more plant biomass (shoot length) than those infected only with *V. dahliae* spores or with *S. sclerotiorum* mycelia suspension (Fig. 3).

4.Conclusions

Olive oil mill wastewater (olive OMW) contains phytotoxic components capable of inhibiting the growth of microorganisms (Ramos-Cormenzana *et al.* 1995) and plants (Martin et al., 2002). Olive OMW contains phenolic compounds (Ramos-Comenzana *et al.* 1995) polysaccharides, lipids, proteins, and a number of monocyclic and polymeric aromatic molecules (Ethaliotis *et al.* 1999) which might exhibit inhibition effects towards some specific microorganism populations. In the current study filtered sterilised olive OMW significantly reduced the growth of *Pythium* spp., *V. dahliae, S. sclerotiorum* and *F. oxysporum* f.sp.

lycopersici. According to D'Annibale *et al.,* (2004) phenolic compounds are the main determinants of the phytotoxic effect of olive residues. Thus, the phenolics of olive OMW used in this experiment had negative effect on all tested fungi *in vitro*. The used for olive OMW sterilization probably removed or destroyed the phenolic compounds from olive OMW solution resulted a same or a better growth media for all tested fungi *in vitro*. Furthermore, the production of the two species of *Penicillium* (*P. italicum and P. digitatum*) and *B. cinerea* spores on fruits inhibited by olive OMW. We assume that the presence of phenolic compounds on olive OMW suppresses fungi reproduction and possible could offer a protection on fruits from post-harvest diseases. Tomato plants infected with *V. dahliae* spores or *S. sclerotiorum* mycelia suspension and treated with olive OMW produced well developed plants compared with the plants infected only with *V. dahliae* spores or *S. sclerotiorum* mycelia suspension. Same results have been reported (Bonanomi *et al.* 2006, Vagelas *et al.* 2009) for olive mill residues affect saprophytic growth and disease incidence of foliar and soilborne plant fungal pathogens same as *Penicillium* spp., *B. cinerea*, *V. dahliae* and *S. sclerotiorum* presented in this research. Overall, we believe that the olive OMW due to phenolics have antifungal activity and could possibly use against soil borne fungal pathogens and fruit parasites such as *Penicillium* spp., causing plant or post-harvest diseases, respectively.

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ANALYSIS AND OPTIMIZATION OF PULSED ELECTRIC FIELD DISTRIBUTION EFFICIENCY IN A CYLINDRICAL TREATMENT CHAMBER FOR JUICE EXTRACTION

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ABSTRACT

Pulsed electric fields (PEF) technology has been receiving wide attention. The PEF treatment has the ability to trigger functional modifications in biological cells, without irreversible disruption of the cell membranes. Indeed, this process depends on several parameters such as the strength, pulses number and pulse duration of pulsed electric field (PEF). However, the influence of pulsed electric field distribution is also one of the key components in the PEF treatment process. The aim of this study to mention the effect of the Electric Field distribution based on Response Surface Modeling (RSM) for identifying the set point of the juice extraction process using pulsed electric field pre-treatment. This parameter was studied by using the different cylindrical treatment chambers built in laboratory. The experiments were carried out on a laboratory experimental bench and the obtained results are very important not only in juice extraction yield, but for quality of final product.

1. Introduction

The pulsed electric field (PEF) technology can be considered as a potential alternative to traditional thermal treatment for food with the advantages of minimizing sensory and nutritional damage, thus providing fresh-like products (Alirezalu *et al.,* 2019; Bobinaitė *et al.,* 2015; Zhang *et al.,* 1995). The technology involves the application of short pulses (microseconds pulse duration) of high voltage to food sample placed between two electrodes. The applied pulse energy destroys the cell membrane, resulting in the creation of pores called the phenomenon of electroporation with minimal heating of the food (Pillet *et al.,* 2016). PEF processing has been successfully used for variety of liquids and pumpable food products such as orange and cranberry juices (Buckow et al., 2013; Dróżdż *et al.,* 2019), and apple juice and cider (Evrendilek *et al.,* 2002; Turk *et al.,* 2012; Xiufang *et al.,* 2013) without any loss of their natural characteristics. It has also been successfully used in enhancing juice extraction from apple, sugar beet, and alfalfa Agcam *et al.,* 2014; Gachovska *et al.,* 2006; Korma *et al.,* 2016; Yeom *et al.,* 2000).

The application of electric fields to biological cells in a conducting medium causes buildup of electrical charges at the cell membrane, and consequently a change in the voltage across the membrane. For low electric fields, this causes voltage-dependent gating, the voltage-induced opening of channels in the cell membrane. A flux of ions through the channels, e.g., sodium and potassium ions changes the ion concentrations close to the cell membrane

and causes cell stress. The stress for short-duration, low-electric-field electrical signals lasts on the order of milliseconds, and does not cause irreparable damage.

At higher electric fields, and a correspondingly higher voltage across the cell membrane, the permeability of the membrane increases to such a level that either the cell needs from seconds to hours to recover (reversible breakdown), or cell death may occur (irreversible breakdown). The mechanism of this membrane breakdown is not well understood. The most common hypothesis is that pores are generated, openings in the membrane of sizes which allow the exchange of macromolecules. Applications of electroporation, the reversible opening of pores which allows for example DNA to enter the cell, are in medicine and biotechnology (Rodrigo et al., 2001). The pores may close again after times which could last hours (Timmerman et al., 2014) or the damage may, at very high fields, become irreparable, and cell death occurs mainly used in bacteriological inactivation treatment (Jemai *et al.,* 2006; Anselmo *et al.,* 2015).

The pulsed electric field method, applied to the food field, consists in subjecting the food to electric fields of very high intensity (5 to 55 kV / cm), repeatedly (pulsed), for very short periods of time (from order of the microsecond), in order to treat the food products they contain. The food product is placed in the treatment chamber, where two electrodes are connected together with a nonconductive material to avoid electrical flow from one to the other.

Nowadays, despite the fact that the treatment chambers currently used give good performances, a good electric field distribution in the treatment chamber remains a major challenge for the PEF technology in order to better treat the food. The main objective of this work is to show that it is possible to give more juice by a better electric field distribution inside the treatment chamber with a good quality of betanine and to validate an experimental procedure for optimizing the extraction process using a laboratory experimental set-up, which was successfully used in other research fields for modeling and optimization (Bellebna *et al.,* 2017; Bermaki *et al.,* 2017).

2. Materials and methods

Fresh beet stems, each of average mass 50 g were obtained at the local market of fruits and vegetables. After sorting and cleaning operations, they were comminuted with a domestic food processor (Thomson, THMX05736 Model) for 5 min to obtain a homogenous mash. The obtained mash was then kept in a closed vessel to prevent evaporation prior to use. Before each experiment, the mash was properly mixed to obtain a homogenous mixture. It was found that the initial moisture content in the mash was of 62% wet basis.

Three cylindrical treatment chambers of different configurations were used in this study, all of which are made of an external electrode of 8 cm in diameter each and differ in terms of the internal electrodes and the gap between the electrodes. The first configuration consists of two concentric cylindrical electrodes (Con1external electrode connected to the ground, an internal electrode of 6 cm diameter connected to the high voltage and a gap between the electrodes of 1 cm (Figure.1). The second configuration is constituted also of two concentric cylindrical electrodes (Con2) an external electrode connected to the ground, an internal electrode connected to the high voltage of 4 cm diameter and a gap between the electrodes of 2 cm (Figure.2). The third configuration is constituted of the same external electrode connected to the ground while the internal electrode connected to the high voltage of 2 cm diameter and a gap between the electrodes of 3 cm (Con3) (Figure.3). In order to increase the processing capacity and provide an intense electric field for a more effective treatment.

After PEF treatment, an extraction step was achieved using an extraction chamber and a hydraulic pressing machine (Mega, 15 tons, Spain).

The chamber for extraction consisted of an insulated cylinder made of plastic (Teflon, PTFE) of length 140 mm and diameter 70 mm (Figure.4), a cylindrical plunger and a disc base of a same diameter 70 mm having a rigid structure for juice pressing operation, both made with stainless steel. Extracted juice was filtered through a stainless steel sieve placed on top of the perforated plunger. Juice extracted during pressing was collected in a plastic collector placed under this chamber. The volume of the treatment chamber was 192.3 ml. For all experiments, the same treatment chamber was used for both pressing step.

The pressure was applied using a hydraulic pressing machine (Mega, 15 tons, according to its labelling). Just after PEF treatment, the filled treatment chamber was pressed until a defined pressure of 50 kg/cm^2 and was then held at this pressure for 5 min.

The PEF treated extracted juice was then analyzed by measuring both its mass using an electronic balance of 0.1 mg precision and the betanine concentration by measuring the absorbance of beet juice using a spectrophotometer (Optizen 200 plus,Mecasys Co., Ltd) at λ= 530 nm.

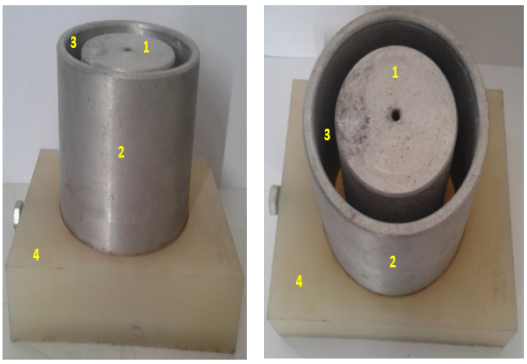


Figure 1. First configuration concentric cylindrical treatment chamber (Con1)
1. Internal electrode, 2. External electrode, 3. Gap between the electrodes, 4. The basis of the treatment chamber

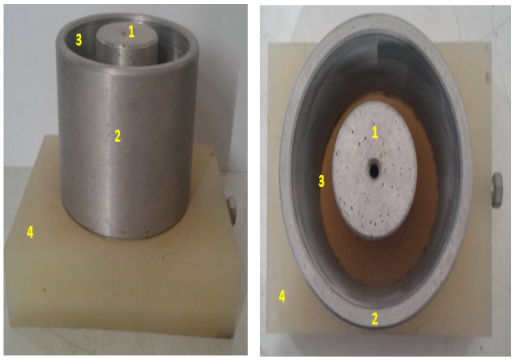


Figure 2. Second configuration concentric cylindrical treatment chamber (Con2)
1. Internal electrode, 2. External electrode, 3. Gap between the electrodes, 4. The basis of the treatment chamber

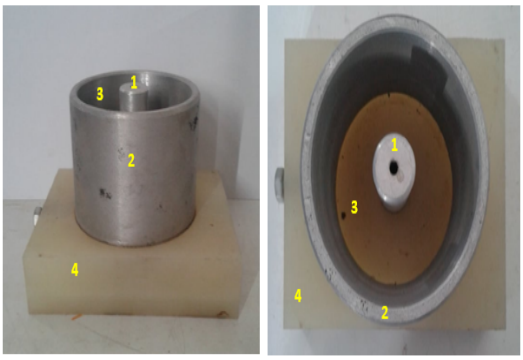


Figure 3. Third configuration concentric cylindrical treatment chamber (Con3)
1. Internal electrode, 2. External electrode, 3. Gap between the electrodes, 4. The basis of the treatment chamber

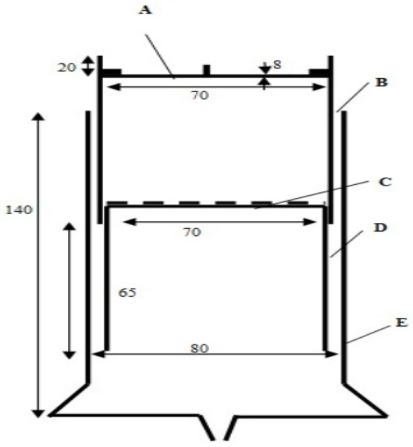
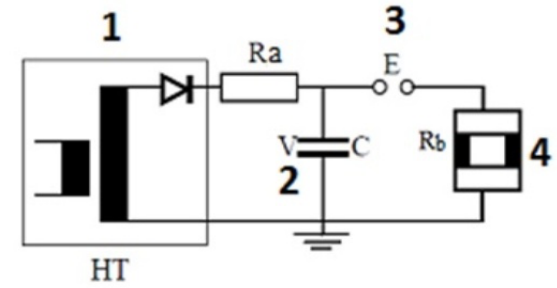


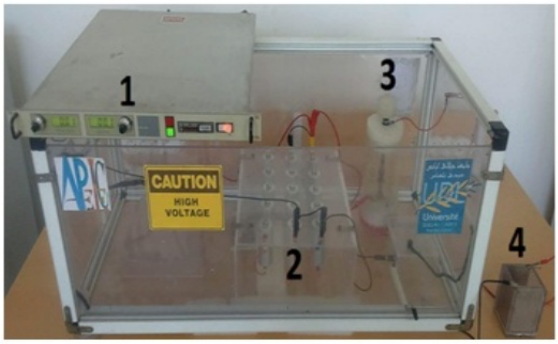
Figure 4. Schematic description of the chamber for extraction step (All dimensions are in mm). A-Stainless Steel disk, B – Teflon cylinder, C – Stainless steel sieve, D -Perforated stainless steel plunger, E- Plastic container for the collection of extracted juice
(Bellebna *et al.,* 2017)

The pulse generator provides electrical exponential decay shape pulses of the desired voltage, and duration. The DC power supply (1) charges the capacitors bank (2) to the determined voltage. Using this device, the AC power from the utility line 50 Hz, is converted in alternating current (AC) high voltage power and then rectified to a DC high voltage power (figure 5). The energy provided by the DC power supply is temporarily stored in the capacitor(s) and then delivered very quickly in form of pulses to the treatment chamber by using the stainless steel spheres of the spark gap discharger of 15 mm in diameter, to generate the necessary electric field strength (figure 5).

A variable autotransformer (AT) (Langlois ALT5A) was used to supply the voltage to the circuit. The input voltage was regulated by the variable autotransformer (AT) to obtain a pulse frequency of 1 Hz, which was kept constant for all the study.



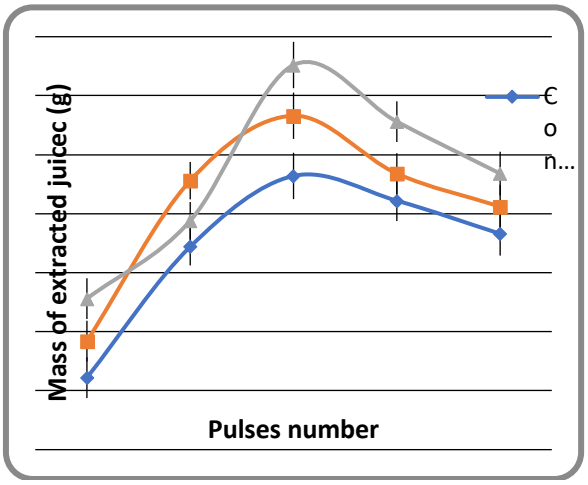
(a)



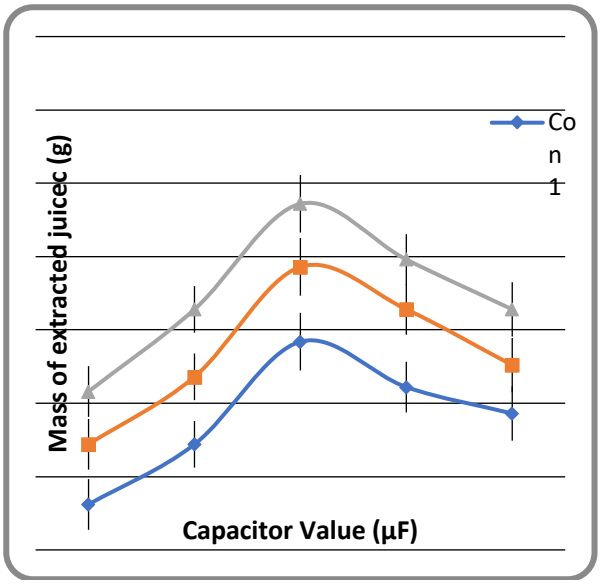
(b)

Figure 5. The pulse generator.
a) Descriptive schematic of the setup;
b) The photography of the setup
1- HV DC power supply, 2-Set of capacitors, 3-Spark gap switch, 4-Treatment chamber

3. Results and discussions

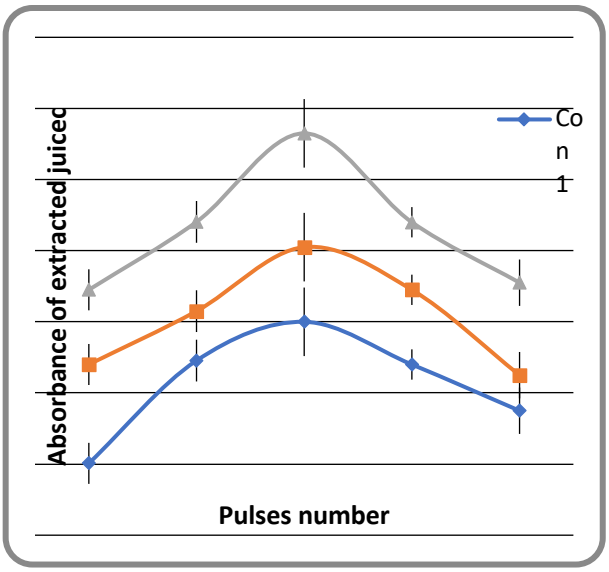


a)

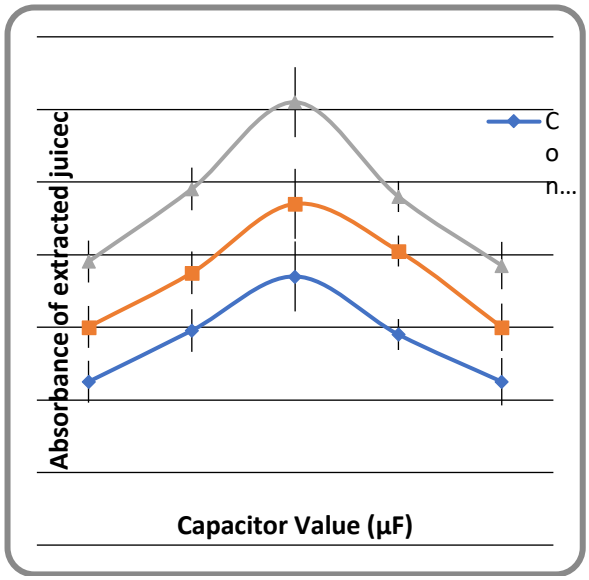


b)

Figure 6. Mass of beet juice extracted for different treatment chamber configuration according to
a) Pulses number (E = 2 kV/cm, C = 0.4 µF),
b) Capacitor value (E = 2 kV/cm, n = 60)



a)



b)

Figure.7. Absorbance of beet juice extracted for different treatment chamber configuration according to
a) Pulses number (E = 2 kV/cm, C = 0.4 µF),
b) Capacitor value (E = 2 kV/cm, n = 60)

For all the experiments carried out in this section, for each configuration model, one factor was varied while the two other factors were kept at constant values. Thus, figure 6 represent the variation of the PEF treatment efficiency, in terms of extracted juice mass (m), according to the pulses number n and the capacitor value C respectively from the same intensity of electric field, which was kept constant for all the study of 2 kV/cm. In order, the electric field depended of input voltage and distance between the electrodes.

In the same way, in Figure.7, is represented the evolution of the absorbance (Abs) as function of n and C respectively.

Initial results shown that, the mass of extracted juice and the quantity of betanin obtained with a PEF-treated sample increases according to the pulses number (Figures 6 and Figure 7), for all models of treatment chambers. Further efficiencies may be obtained by using Con3 treatment chambers model compared with the Con1 and Con2.The PEF efficiency can be minimized if the application of pulses number or capacitor value exceeds a determined value, the effect of the PEF treatment is inversed due to an excess energy provided to the product, which causes the opposite effect.

When the diameter of the electrode of the high voltage is low the electric field became more intense due to the dissymmetry in the electrodes of the treatment chamber which makes it possible to penetrate the electric field lines in the deep layers of the tissue to be treated leads to an effective treatment by pulsed electric field.

As well as the yield of extracted juice is significant, this distribution of the electric field gives the possibility of treatment of a large mass because the distance offered by this configuration of the treatment chamber is greater.

Increasingly the diameter of the active electrode increases the juice yield decreases. This is because pulsed electric field lines have become unable to penetrate deeply into the tissue of the food and only treat the surface of food because the electric field in this case has become lower with a quasi-uniform distribution.

The electroporation process is more efficient in Con3 compared with the order treatment chambers

3.1. Experimental designs methodology

Methodology of the experimental designs makes it possible to determine the number of experiments to be achieved according to a well defined objective, to study several factors simultaneously, to reduce dispersion related to measurements, to appreciate the effects of coupling between factors and finally to evaluate the respective influence of the factors and their interactions son

The Composite Centred Faces design (CCF), which gives quadratic models, was adopted. A quadratic dependence is established between the output function to optimize (response) and the input variables (Cheng *et al.,* 2016; Moradi *et al.,* 2016; Swamy *et al*., 2014).

MODDE 5.0 software (Umetrics AB, Umea, Sweden) was used, which is a Windows program for the creation and the evaluation of experimental designs (MODDE 5.0., 1999).

The three following factors are studied:

1. Number of pulses n;
2. Capacitor value C (µF);
3. Treatment chamber Configuration (Coni) models are defined by 1, 2 and 3 for Con1, Con2 and Con3 respectively.

Indeed, Obtain results in previous section served to the definition of the domain of variation of n, C and Con_i. Thus, n_{min} = 40 and n_{max} = 80 were retained as the limit values for pulses number.

In the same for capacitor value, the domain of variation was chosen as C_{min} = 0.4 µF and C_{max} =1.2 µF. Indeed, we opted for the treatment chamber configuration model as Con_{min}= 1 and Con_{max}= 3 as limits of variation domain of Con.

The results of all the experiments are given in Table 1 and Figures 8 - 11 served to define the domain of variation of n, C and Con to indentify a mathematical model using MODDE 5.0 software.

According to all of the experiments, modeling software MODDE 5.0 gave us a mathematical model of juice extraction and absorbance using pulsed electric field treatment. This mathematical model is very satisfactory because the coefficients R^2 and Q^2 are very close to 1(figure 8). MODDE 5.0 also gives the effect of each parameter on extracted juice yield and absorbance value (figure 9).

Table 1. Results juice mass and absorbance experience extract according to variation in treatment values

Exp No	*n*	*C* [µF]	*Con*	*Masse of juice* [g]	*Absorbance*
1	40	0.4	1	23.3	0.831
2	80	0.4	1	26.4	1.087
3	40	1.2	1	31.8	1.015
4	80	1.2	1	31.7	0.51
5	40	0.4	3	33.7	0.288
6	80	0.4	3	42.3	0.377
7	40	1.2	3	37.3	1.01
8	80	1.2	3	40.4	0.32
9	40	0.8	2	30.7	0.796
10	80	0.8	2	33.1	0.514
11	60	0.4	2	37.5	0.439
12	60	12	2	37.1	0.421
13	60	0.8	1	33	1.149
14	60	0.8	3	41	0.565
15	60	0.8	2	37.2	0.503
16	60	0.8	2	37.2	0.503
17	60	0.8	2	37.2	0.503

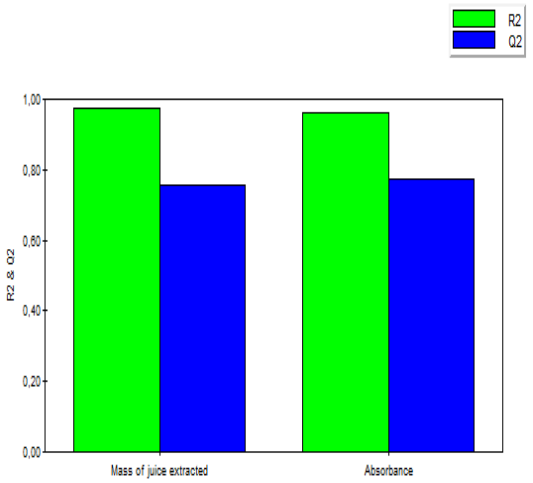


Figure 8. Representation of descriptive quality and predictive quality of mathematical model of juice extraction and absorbance

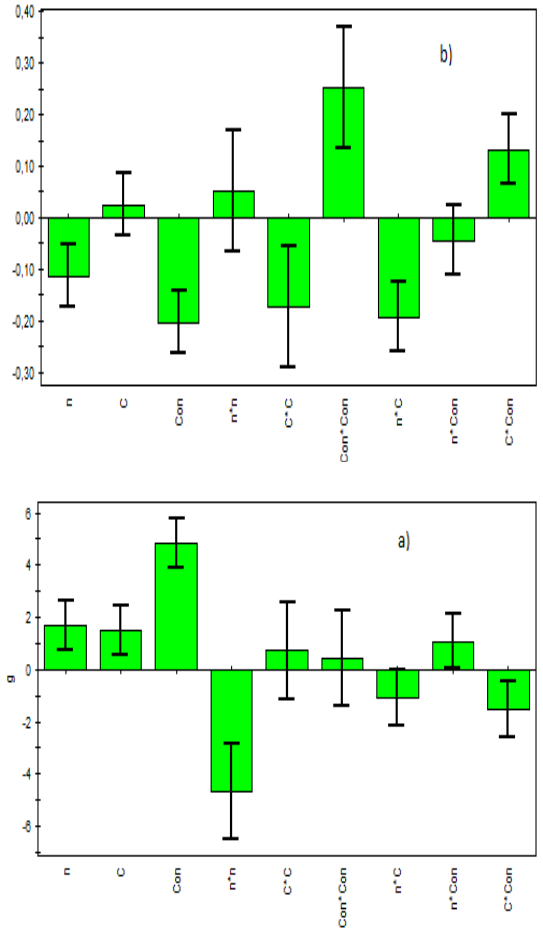


Figure 9. Values of the influence of each factor and their interactions
a) Mass of juice extracted, b) Absorbance

The predictive qualities of the model are satisfactory since the coefficient values Q^2 and R^2 close to 100% (R^2 = 0.97, Q^2 = 0.76 for juice extracted and R^2 = 0.97, Q^2 = 0.78 for absorbance) lead to a validated mathematical model (figure 8).

The mass of extracted juice M and absorbance ABS are the responses of the experimental design; the mathematical models were obtained as follows:

$$M = 36{,}84 + 1.71\ n^{*} + 1.51\ C^{*} + 4.85\ Con^{*} - 4.68\ n^{*2} - 1.08\ n^{*}C^{*} + 1.08\ n^{*}Con^{*} - 1.51\ C^{*}\ Con^{*} \quad (1)$$

$$Abs = 0.560 - 0.11\ n^{*} - 0.2\ Con^{*} - 0.17\ C^{*2} + 0.25\ Con^{*}2 - 0.19\ n^{*}\ C^{*} + 0.13\ C^{*}Con^{*} \quad (2)$$

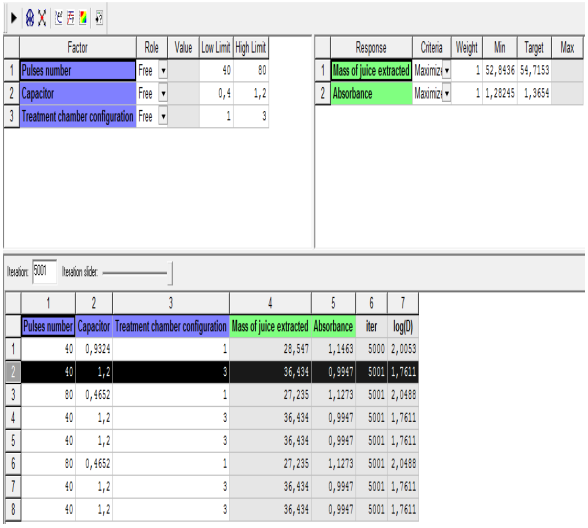
According to the mathematical model, the configuration of treatment chamber is the most important and influential factor in the mass yield of extracted juice and absorbance. This too is evident in these interactions with other factors.

The mass of the extracted juice should be higher by using the appropriate configuration of treatment chamber both the pulses number n and the capacitor value.

Moreover, except the interaction between the pulses number and the configuration with the capacitor value, other interactions are not significant. This means that the delivered energy during one pulse has an important effect on the electroporation process (figure.9-a).

On the other hand, the configuration of the treatment chamber has a negative influence on the absorbance that is explained that the absorbance decreases while increasing the amount of extracted juice (figure.9-b).

According to this model, the optimum of the process (i.e., the greatest amount of beet juice and absorbance) should be obtained for number of pulses n0 = 40, capacitor value C_0 = 1.2 µF and with using of the third treatment chamber configuration Con = 3 (figure 10).



	Factor	Role	Value	Low Limit	High Limit
1	Pulses number	Free		40	80
2	Capacitor	Free		0,4	1,2
3	Treatment chamber configuration	Free		1	3

	Response	Criteria	Weight	Min	Target	Max
1	Mass of juice extracted	Maximize	1	52,8436	54,7153	
2	Absorbance	Maximize	1	1,28245	1,3654	

Iteration: 5001 Iteration slider:

	1	2	3	4	5	6	7
	Pulses number	Capacitor	Treatment chamber configuration	Mass of juice extracted	Absorbance	iter	log(D)
1	40	0,9324	1	28,547	1,1463	5000	2,0053
2	40	1,2	3	36,434	0,9947	5001	1,7611
3	80	0,4652	1	27,235	1,1273	5001	2,0488
4	40	1,2	3	36,434	0,9947	5001	1,7611
5	40	1,2	3	36,434	0,9947	5001	1,7611
6	80	0,4652	1	27,235	1,1273	5001	2,0488
7	40	1,2	3	36,434	0,9947	5001	1,7611
8	40	1,2	3	36,434	0,9947	5001	1,7611

Figure 10. Subroutine of MODDE.05 representing the set point

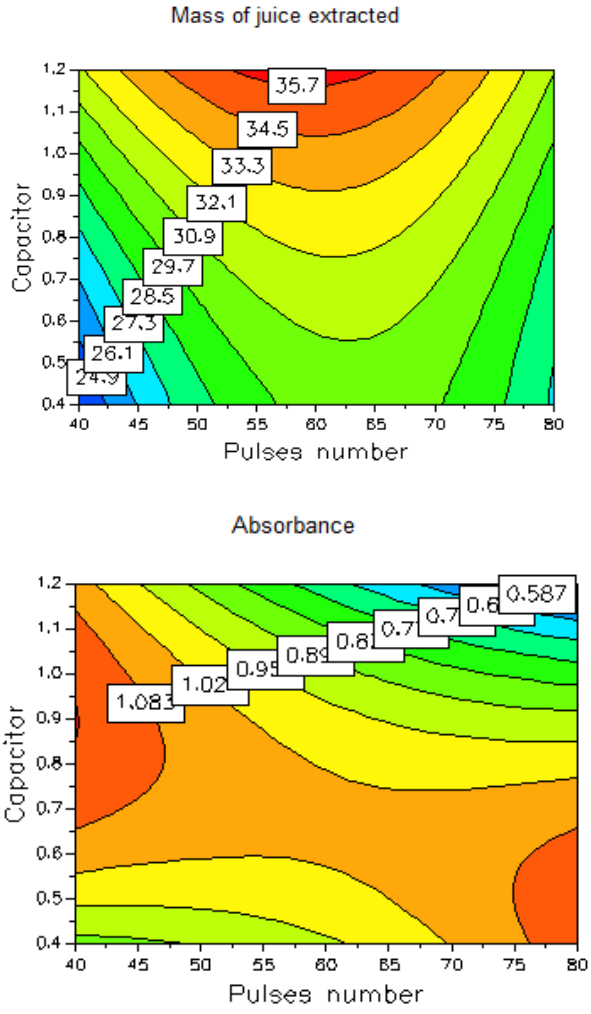


Figure. 11. Response contour plots of the masse of juice extracted and absorbance for Con1

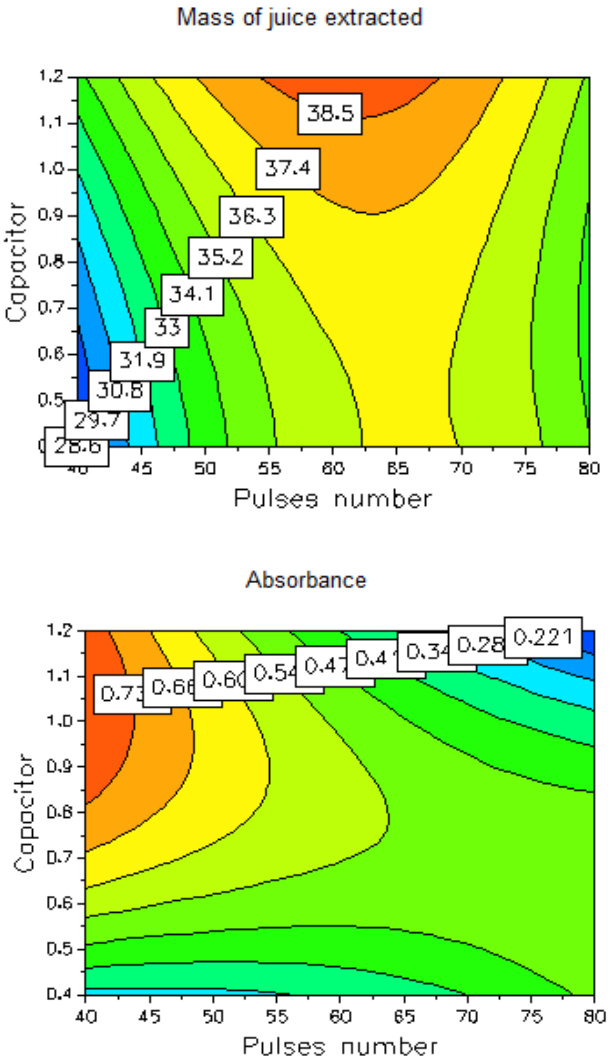
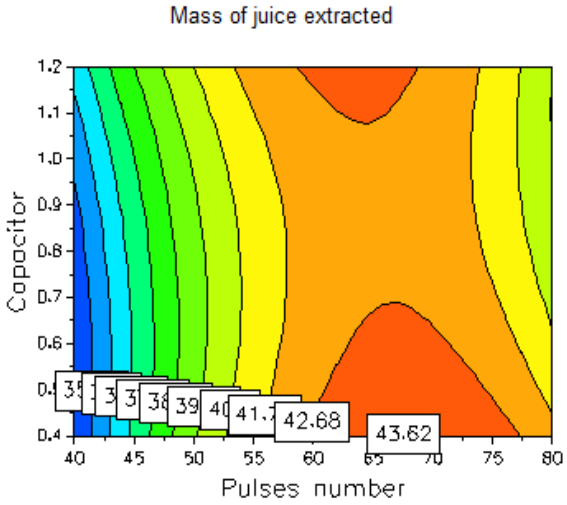


Figure. 12. Response contour plots of the masse of juice extracted and absorbance for Con2



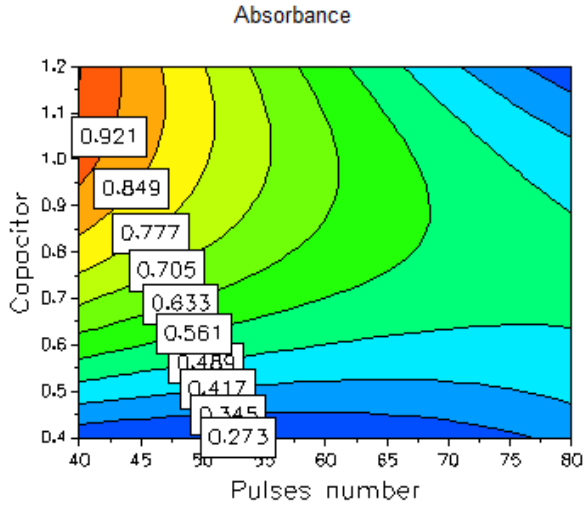


Figure. 13. Response contour plots of the masse of juice extracted and absorbance for Con3

4. Conclusions

In conclusion, results obtained in this investigation confirm that the applications of PEF treatments in the range of asymmetrical treatment chamber configurations are more effective in the enhancement of the beet juice extraction. However, treatments delivered in the range of treatment chambers of symmetrical or quasi-symmetrical treatment chamber configurations were more effective in the extraction of betanine.

Finally, in addition to the proper choice of the treatment chamber configuration the pulses number requirements and the capacitors value processing required for PEF process are also significant parameters for an effective treatment.

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THE EFFECT OF BUCKWHEAT FLOUR ON SOME QUALITY PROPERTIES OF CHICKEN MEATBALLS AS AN ALTERNATIVE TO WHEAT FLOUR

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ABSTRACT

Chicken meatballs were produced by using mixtures at different ratios of wheat flour and buckwheat flour. Fried meatballs were analysed about yield, diameter reduction, colour, moisture retention, fat absorption and sensory properties. Cold stored raw meatballs were evaluated for pH, Thiobarbutiric acid reactive substances and instrumental colour values. Addition of buckwheat flour in meatballs increased the yields and L^* values after frying whereas decreased the diameter reductions, a^* and b^* values. Also, it increased the moisture retention and decreased fat absorption, had high scores at low levels of flour for colour and texture. pH, L^*, a^* and b^* values of raw samples were better at low ratios of buckwheat flour at the end of the storage whereas Thiobarbutiric acid reactive substances decreased with the increase of buckwheat flour. In view of all the results, it can be said that the use of 1:3 or 3:1 buckwheat flour: wheat flour mixes in the production of chicken meatballs would be more advantageous.

1. Introduction

The demand for ready-to-eat foods increases with the intensity of daily living conditions in societies. In particular, working people and young people demand more of this type of food. Therefore, food manufacturers try to develop products that meet this kind of food needs of consumers. They aim to win consumers' appreciation by producing food that is easy to prepare and even suitable for healthy nutrition (Dani and Pabalkar, 2013; Ivankovic et al., 2017; Özer and Seçen, 2018).

In order to meet the requirements mentioned in the prepared product, various food additives or natural sources which may contain these substances are used. Examples of these are structure modifiers such as starches, fibres, proteins, or preservatives such as organic acids or sensory quality enhancers such as colorants. Other examples are the use of flours of plants such as wheat, corn, chickpea or quinoa, which may contain some of the substances mentioned. The structural quality of the product can be preserved by using these flours and the shelf life can be increased. Different alternatives can be created for the healthy nutrition of consumers by increasing the fibre ratio in the product with the use of flours. Also, while the properties of foods such as colour and texture can be improved, the yield and moisture content after cooking can be increased due to the hydrophilic properties of the flour components, and the absorbed fat content can be reduced (Tarte, 2009; Petracci et al., 2013).

Poultry products are the most common groups where additives or flours are used. The quality of these products can be influenced by the using of mentioned materials. The delicate

structure of these meats can be supported by different vegetable flours, which can be advantageous in processing, preservation and consumption. Disintegration and oxidation problems in chicken meats can be reduced and colour can be improved (Dogan et al., 2005; Petracci et al., 2013; Santhi and Kalaikannan, 2014).

While the use of many plant-based flours in food preparation can be emphasized, another alternative is the buckwheat flour. Buckwheat is rich in vitamins, minerals and starches and is particularly advantageous in terms of protein and fibre content compared to wheat. It even has a protein structure with high biological value and does not contain gluten. Additionally, there are studies indicating that it is rich in antioxidant compounds such as polyphenols (Przybylski and Gruczynska, 2009; Wronkawska et al., 2010).

For these reasons, it is thought that it can provide various benefits in meatball production. However, it is understood that there is not much work done by adding buckwheat flour to chicken meatballs. Therefore, in this study, the effect of buckwheat flour mixed with wheat flour on some quality properties of chicken meatballs was investigated.

2. Materials and methods

Wheat flour (W), buckwheat flour (B), chicken meats and other ingredients were purchased from local sellers in Adiyaman and Istanbul (Turkey). Sunflower oil (Yudum, Balıkesir, Turkey) and mini fryer (Tefal, FF1024, China) were used for frying operations. The chicken breasts were freshly taken and kept at 4 ºC until the meatballs were produced, then they were minced in mincing machine for production. Five mixes were prepared as 100% W, 1: 3 B: W, 1: 1 B: W, 3: 1 B: W and 100% B. Then, samples were produced with 90.5% minced meat, 7% mix, 1.5% salt and 1% sunflower oil. Each of the samples was thoroughly kneaded and allowed to stand for 20 minutes at 4 ° C. Then, they were turned into round meatballs weighing 19 g and having a diameter range of 31-32 mm. Some of the meatballs were used in frying processes whereas the others were used for storage stability analyses. The first group was fried for 6 min at 180 ºC and their yield, diameter reduction, colour, moisture retention, oil absorption values and sensory quality characteristics were determined. The second group was stored at 4 ºC and changes in pH, TBARS and colour values were determined after 1, 5 and 10 days of storage.

2.1. Determination of some physical and chemical properties of flours

The colour values of flours were determined by a colorimeter (CR-400, Konica Minalto, Inc., Osaka, Japan). Water absorptions were determined according to Dogan and Unal (1990). The moisture contents of flours were founded gravimetrically by oven-drying (Nuve, FN500, Turkey) at 105 ºC for 4h, while the protein contents were detected by using Kjeldahl analiysis (Behr S2, Germany; AOAC, 2002). Total starch contents of flours were determined by using polarimeter (Autopol V Plus, USA), according to Elgün et al. (1998). From the nitrogen to protein conversion factor was applied as 5.7 for flours whereas the conversion factor of starch was used as 5.4734.

2.2 Determination of the yield, diameter reduction and colour values

The following equations (1) and (2) were used to calculate frying yields and diameter reductions. A precision balance was used for weight measurements, while a digital calliper (Mesem, Turkey) was used for diameter measurements (Kırpık and Kılınççeker, 2018).

$$\text{Frying yield} = \frac{\text{fried meatball weight}}{\text{raw meatball weihgt}} \text{ x } 100 \quad (1)$$

$$\text{Diameter reduction (\%)} = \frac{\text{raw meatball diameter} - \text{fried meatball diameter}}{\text{raw meatball diameter}} \text{x} 100 \quad (2)$$

The colour values of the fried samples and the raw samples in storage were determined by

a colorimeter (CR-400, Konica Minalto, Inc., Osaka, Japan) with illuminate D65, 2° observer, Diffuse/O mode, 8-mm aperture of instrument for illumination and 8 mm for measurement. The colorimeter was initially calibrated with a white references tile. After the measurements, color values were presented according to CIELAB system as L^* (brightness), a^* (redness) and b^* (yellowness) values (Dogan, 2006). The colour of fried meatballs was measured in 4 min after the frying whereas it was measured in the end of each storage period for raw samples. Four meatballs were taken for each treatment and colour measurement was performed at three different points on surface.

2.3. Determination of moisture retention, fat absorption and sensory properties

Equations (3) and (4) were used to calculate moisture retention and oil absorption (Soltanizadeh and Ghiasi-Esfahani, 2015). An oven (Nuve, FN500, Turkey) at 105±2 °C temperature was used to find the moisture content of the raw and fried samples, while soxhlet extraction method with n-hexane was used to find the oil contents (Gerhardt, Germany; AOAC, 2002).

$$\text{Moisture retention (\%)} = \frac{\text{moisture in fried meatball (\%)}}{\text{moisture in raw meatball (\%)}} \text{ x frying yield} \quad (3)$$

$$\text{Fat absoption} = \text{fat in fried meatball (\%)} - \text{fat in raw meatball(\%)} \quad (4)$$

Ten students (five female and five male) from the food processing department of Adiyaman University were selected for sensory analysis of fried samples. The panellists evaluated on the hedonic scale for appearance, colour, smell, taste and texture. The method contained the scores in the range of 1 and 9 which depending on the degree of liking (1: dislike very much, 9: like very much), as described in Gokalp et al. (1999).

2.4. Determination of pH and TBARS

These analyses were performed on raw samples to obtain information about storage stability in the end of each storage period. For pH analysis, 10 g of minced sample was homogenized in 100 ml of deionized water for 3 minutes and was measured by a pH meter (Orion 3-Star, Thermo Fisher Scientific, Waltham, M.A), as described by Ockerman (1985). TBARS analysis were determined according to the Tarladgis et al. (1960). For this analysis, 10 g of minced sample was mixed with 50 ml of distilled water. After the mixture, the homogenized sample was transferred to an 800-ml kjeldahl flask and 47.5 ml of distilled water and 2.5 ml of 4 N HCL were added on it. Then 50 ml of distillate were obtained from this mixture in the distillation unit. 5 ml of distillate was transferred to the tube and 5 ml of TBA reagent was added. It was heated for 35 minutes in boiling water and the absorbance at 538 nm was read using the spectrophotometer (UV-160 A, UV-Visible Recording Spectrophotometer, Shimadzu, Tokyo, Japan). TBARS numbers were multiplied by a predetermined coefficient (7.8) and expressed as mg malonaldehyde/ kg sample.

2.5. Statistical analysis

The study was conducted in two replications and three parallels. The data were subjected to analysis of variance (ANOVA). When there was difference between the samples, Duncan multiple comparison test at level of $p<0.05$ was applied to compare of mean (SPSS 16.0, CHICAGO, IL, USA). The results were presented as mean±standart deviation.

3. Results and discussions

3.1. Some properties of flours

Some physical properties of flours such as colour and water absorption and chemical properties such as moisture, protein and starch have a significant effect on product quality when they are added to foods such as meatballs. In this study, it was thought that these properties of the wheat and buckwheat flours will be helpful in the discussion of the results. According to the

results shown in Table 1, while *L** and *b** values for B were lower than W, the *a** value and water absorption were higher. In addition, it was determined that the moisture and starch contents of the B were lower than W, the protein content was higher.

Table 1. Some properties of wheat flour and buckwheat flour

	*L**	*a**	*b**	Water absorption ration (%)	Moisture (%)	Protein (%)	Starch (%)
Wheat flour	96.65	-1.47	8.78	65.07	8.22	10.48	72.18
Buckwheat flour	90.91	0.1	6.86	114.21	7.42	11.46	68.15

3.2. The yield, diameter reduction and colour values of fried meatballs

The decrease in yield and diameter after cooking are generally a result of protein denaturation by heat treatment. These are important factors for product quality and can be influenced by the materials in the flour composition. Colour change is also affected by heat treatment, while the type and amount of natural colour pigment of the materials used are important criteria (Cava *et al*., 2012). In table 2; the yield, reductions in diameters and colour values of samples are shown after frying. According to the results, it was found that the addition of buckwheat flour increased the yield and caused an increase in the diameters as can be seen from the (-) signs in the results ($p<0.05$). Especially, the yield and diameter values of the samples containing 3:1 B:W and 100% B mixtures were found to be higher than the others. It is thought that the increase in the yield and diameter of the meatballs is due to the low moisture content of the buckwheat flour and the high protein and fibre ratio in its structure. Proteins and fibres having hydrophilic character caused an increase in weight by keeping water in the structure during frying. They even increased the efficiency by preventing the rupture of the pieces, and the diameters by swelling with heat treatment. Similarly, Ikhlas et al. (2011) observed that the yields increased after cooking when adding different flours to quail meat. Also, Santhi and Kalaikannan (2014) determined that addition of oat flour in chicken nuggets increased cooking yields whereas Kurt and Kılınççeker (2012) found increase of yields in meat patties prepared with different flours. Kırpık and Kılınççeker (2018) found that although there was no statistical difference in the fried chicken meatballs made by adding quinoa flour, the reductions of diameter decreased with increasing amount of flour. Serdaroglu (2006) determined that diameter reductions of cooked meat patties produced with oat flour decreased with the increase of flour. Also, Soltanizadeh and Ghiasi-Esfahani (2015) observed that diameter reductions of cooked beef burger significantly decreased with increasing of aleo vera powder in formula.

The use of 100% buckwheat flour caused an increase in *L** values ($p<0.05$) whereas *a** and *b** values decreased with the addition of this flour ($p<0.01$). The highest *L** value was in sample with 100% B. The best results for *a* value were found in the control and samples containing 1:3 B:W, while for *b** was found in the control group (Table 2). The natural colour components of the buckwheat flour were effective on the colour of fried meatballs. Although this flour is redder in colour, *a** and *b** values decreased due to the loss of phenolic compounds, which are highly present in buckwheat flour, and which have the ability to colour, during frying. Therefore, the excessive loss of phenolic compounds increased with the addition of this flour caused the brightness of the colour to increase. Lee et al. (2018) observed the increasing of *L** values of cooked sausage produced with buckwheat powder whereas decreasing of *a** value. Park et al. (2016) determined that *b** values of cooked pork patties decreased with buckwheat. Serdaroglu (2006)

was found that *L** values increased and *a** values decreased in beef patties with oat flour. In another study, it was emphasized that the colour values of the meatballs produced by adding different flours to the quail meat were different and the colour pigments in the flours played an important role in this (Ikhlas et al., 2011). In our study, it is understood that colour values are affected by heat treatment as well as pigments.

Table 2. Effects of buckwheat flour on yield, diameter reduction, and colour values of fried chicken meatballs

Flour Mixes	Yield (%)	Diameter reduction (%)	*L**	*a**	*b**
Control	79.91±0.39^{c}	0.51±0.66^{a}	54.35±1.26^{b}	15.73±0.53^{a}	39.08±0.18^{a}
1:3 B:W	82.39±0.82^{bc}	-0.61±0.83^{ab}	55.07±2.24^{b}	15.76±1.02^{a}	38.25±0.47^{b}
1:1 B:W	82.31±1.31^{bc}	-1.01±0.01^{abc}	55.68±0.11^{b}	13.31±0.40^{b}	37.39±0.27^{c}
3:1 B:W	83.99±2.25^{ab}	-1.71±0.23^{bc}	56.86±1.33^{b}	12.07±0.52^{b}	37.11±0.05^{c}
100% B	85.74±0.17^{a}	-2.60±1.18^{c}	61.45±0.31^{a}	8.07±0.03^{c}	35.07±0.26^{d}

W: wheat flour, B: buckwheat flour, $^{a-d}$ Within each column, different superscript letters show differences between the flour mixes ($P< 0.05$).

3.3. The moisture retention, fat absorption and sensory properties of fried meatballs

While moisture and fat contents in foods affect sensory properties such as texture, they also have an importance on calorie value. These are also important features in fried meatballs and affect consumers' preferences. In general, the moisture content of such products decreases, the oil content increases, thus manufacturers turn to various production techniques or additives to solve this problem (Pinero et al., 2008.) In our study, it was determined that adding buckwheat flour increased moisture retention, decreased oil absorption during frying (Figure 1). While the highest moisture retention ratios were determined in meatballs prepared with 3:1 B:W and 100% B mixes as 87.2% and 87.78% ($p<0.05$), the lowest oil absorptions were also observed in these two samples as 0.94% and 0.93% ($p<0.01$). In particular, it is believed that buckwheat flour's water absorption ability, protein and fibre content were effective on these results. These components increased the water absorption ability in the meatballs whereas reduced the water loss and the oil penetration. Ikhlas et al. (2011) found that the addition of different flours in meatballs prepared from quail meat increased ratios of moisture retention during cooking. Serdaroglu (2006) determined that oat flour increased the moisture retention in cooked patties. In addition, in another study, it determined that the increase in amount of flour in chicken meatballs prepared by adding quinoa flour increased moisture retention and decreased fat absorption during frying (Kırpık and Kılınççeker, 2018). Similarly, Soltanizadeh and Ghiasi-Esfahani (2015) found that addition of aloe vera powder to meat burgers in the range of 1-5% increased the moisture retention and decreased the fat absorption during frying. They also observed that the increase in the amount of aloe vera supported the results. In these studies, it was said that components such as protein, starch and fibre in the structure of plants are effective on the results. Especially, in studies with flours, it was emphasized that strong structures which were formed by coagulation of proteins and gelatinization of starch were effective against substance transfer. Our results were similar to these works.

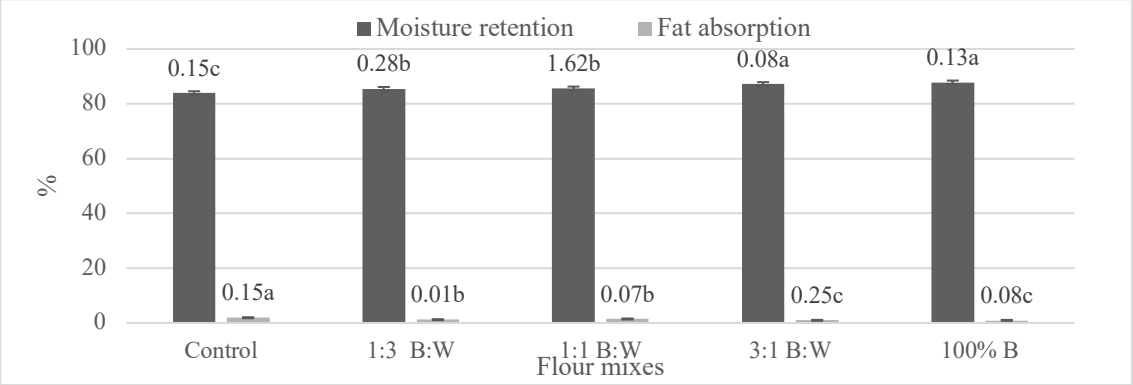

Figure 1. Effects of buckwheat flour on moisture retention and fat absorption rations of fried chicken meatballs

Sensory characteristics are the most important criteria on consumer choice. These properties can be influenced by the type and amount of ingredients used in foods. They should be identified for newly developed products (Soltanizadeh and Ghiasi-Esfahani 2015; Kırpık and Kılınççeker, 2018). In our study, colour and texture properties were affected from buckwheat flour ($p<0.05$). While 100% buckwheat flour decreased these properties as 5.75 and 5.95 ($p<0.05$), it was found that the other mixtures were not different statistically and scored high (Table 3). The decrease in colour scores can be attributed to the decrease in red and yellow colour ratios, as can be seen from the measurements with the colorimeter. Because, these colours are the criteria's that support the desired golden-red (or golden-yellow) colour formation in fried products. The decrease in texture scores is thought to be due to the hard structure caused by excessive amounts of buckwheat flour. This firmness also emphasized by the panellists. Similar to the results, Kılınççeker and Karahan (2019) determined that the colour and texture scores in chicken meatballs prepared with adding of carob flour and breadcrumbs were high such as in control but decreased when 100% carob flour was used. In addition, Kırpık and Kılınççeker (2018) found that when the mixtures prepared with quinoa flour and breadcrumbs were added in chicken meatballs, the colour scores of other mixes were not different, but scores of samples with 100% carob flour also decreased, statistically. In addition, the texture scores were found to be at acceptable level and statistically not different in this study. Finally, Santhi and Kalaikannan (2014) observed that when the addition of oat flour in chicken nuggets, many quality characteristics were improved, however some of sensory scores decreased.

Table 3. Effects of buckwheat flour on sensory properties of fried chicken meatballs

Flour Mixes	Appearance	Colour	Odour	Taste	Texture
Control	6.90±0.28^{a}	6.65±0.21^{ab}	6.15±0.21^{a}	6.95±0.49^{a}	7.25±0.07^{a}
1:3 B:W	6.65±0.49^{a}	7.05±0.07^{a}	6.80±0.14^{a}	7.05±0.21^{a}	7.15±0.07^{a}
1:1 B:W	6.95±0.64^{a}	7.35±0.21^{a}	6.20±0.28^{a}	6.85±0.92^{a}	6.75±0.64^{ab}
3:1 B:W	6.75±0.49^{a}	6.80±0.42^{a}	6.40±0.85^{a}	7.20±0.56^{a}	7.00±0.28^{a}
100% B	6.10±0.71^{a}	5.75±0.64^{b}	5.70±0.99^{a}	5.85±0.78^{a}	5.95±0.07^{b}

W: wheat flour, B: buckwheat flour, $^{a-b}$ Within each column, different superscript letters show differences between the flour mixes ($P< 0.05$).

3.4. The pH, TBARS and colour values of raw meatballs

Protolithic and oxidative degradation in sensitive foods such as meatballs are the most important factors affecting product quality. Storage time has effects on these. During storage, substances that occur such as ammonia increase the pH value, while products such as aldehyde formed as a result of oxidation of fatty acids increase the level of TBARS. Thus, smell deteriorates, and taste may be bitter. Therefore, both pH and TBARS can be used as indicators of raw meatball quality. Even colour values may be affected by them. The materials in formulas can affect the pH, TBARS and colour of the raw meatballs. Thus, it is recommended to measure these values periodically (Gokalp et al., 1999). In general, buckwheat flour and storage times had an effect on pH and TBARS values (Table 4). On the first day, the lowest pH was determined as 5.85 in the control, while it was determined in the range of 5.90-5.95 in the other samples (<0.05). The pH values of some samples decreased after 10 days of storage. At the end of the 10th day, the lowest pH values were measured as 5.73, 5.77 and 5.77 in control and in samples with 1:3 B:W and 1:1 B:W mixes ($p<0.01$). The lowest TBARS value in first day was in control as 0.10 mg/kg. Although this value decreased in some samples on the 5th storage day, it was found that there was generally an increase at the end of 10 days storage. At the end of the 10th day, TBARS values of samples prepared with 3:1 B:W and 100% B mixtures were found to be lower than the others as 0.16 mg/kg and 0.17 mg/kg ($p<0.05$). *L** values decreased with increasing amount of B in meatball content in each storage period. While this value was not generally affected by the increase in storage time, a fluctuating decrease was observed in samples containing only 3:1 B:W. While the highest *L** value was determined as 68.83 in the control sample on the first day, the values of control and samples with 1:3 B:W mix on the 10th day were higher than the others as 68.12 and 67.02 ($p<0.01$). The *a** values of the samples decreased only with the increase of B on the 5th day ($p<0.01$), while these values decreased with the increase of storage time, generally. At the end of 5 days storage the best results were 3.98, 3.81 and 3.83 in the control sample and in the samples prepared with 1:3 and 1:1 ($p<0.01$). The *b** values decreased with increasing B in the end of the 5th and 10th days, while storage time decreased this value of some samples. The *b** values on the first day were in the range of 17.38-19.38. At the end of 10 days of storage, the *b** values of the control sample and samples containing 1:3 B:W and 1:1 B:W mixes were found to be better than others (as 19.19, 18.37 and 17.90).

Table 4. Effects of buckwheat flour on pH, TBARS (mg malonaldehyde/kg sample) and colour values of raw chicken meatballs at cold storage periods

Storage days	Flour mixes	pH	TBARS	*L**	*a**	*b**
1st	Control	5.85±0.00^{bx}	0.10±0.01^{by}	68.83±1.63^{ax}	4.11±0.39^{ax}	19.38±0.22^{ax}
	1:3 B:W	5.90±0.03^{abx}	0.16±0.00^{ax}	66.89±1.89^{abx}	3.99±0.71^{ax}	18.52±1.75^{ax}
	1:1 B:W	5.91±0.01^{aby}	0.19±0.04^{ax}	66.49±1.67^{abx}	3.87±0.27^{ax}	18.71±0.85^{ax}
	3:1 B:W	5.92±0.03^{abx}	0.16±0.00^{ax}	66.39±0.40^{abx}	4.21±0.13^{ax}	18.13±0.06^{ax}
	100% B	5.95±0.01^{ax}	0.18±0.00^{ax}	63.77±1.90^{bx}	4.31±0.20^{ax}	17.38±0.42^{ax}
5th	Control	5.91±0.05^{ax}	0.11±0.00^{ay}	67.45±0.53^{ax}	3.98±0.15^{ax}	19.73±0.27^{ax}
	1:3 B:W	5.90±0.01^{ax}	0.11±0.00^{ay}	67.35±0.11^{ax}	3.81±0.04^{ax}	19.85±0.53^{ax}
	1:1 B:W	5.94±0.01^{ax}	0.09±0.00^{by}	66.35±1.70^{abx}	3.83±0.37^{ax}	19.08±0.25^{ax}
	3:1 B:W	5.92±0.04^{ax}	0.09±0.00^{by}	64.65±0.02^{bcy}	2.93±0.20^{by}	17.86±0.06^{by}
	100% B	5.95±0.03^{ax}	0.09±0.00^{by}	62.92±0.81^{cx}	2.95±0.07^{by}	16.38±0.51^{bx}

10th	Control	5.73±0.00^{cy}	0.22±0.00^{ax}	68.12±0.85^{ax}	2.40±0.56^{ay}	19.19±0.59^{ax}
	1:3 B:W	5.77±0.02^{bcy}	0.19±0.02^{abx}	67.02±0.37^{abx}	2.17±0.10^{ay}	18.37±0.79^{abx}
	1:1 B:W	5.77±0.01^{bcz}	0.19±0.01^{abx}	65.06±0.84^{cdx}	2.11±0.03^{ay}	17.90±0.79^{abx}
	3:1 B:W	5.83±0.05^{bx}	0.16±0.01^{bx}	65.53±0.30^{bcxy}	2.09±0.21^{az}	17.49±0.07^{bz}
	100% B	5.92±0.01^{ax}	0.17±0.01^{bx}	63.65±0.76^{dx}	2.72±0.10^{ay}	17.21±0.50^{bx}

W: Wheat flour, B: Buckwheat flour. $^{a-d}$ Within each column, different superscript letters show differences between the flour mixes within each storage period ($P< 0.05$). $^{x-z}$ Within each column, different superscript letters show differences between the storage periods with respect to same flour mix ($P< 0.05$)

Similarly, Tamsen et al. (2018) studied the effect of using amaranth flour instead of wheat flour in chicken nugget. They mixed the amaranth flour into the wheat flour in 0%, 50% and 100% ratios and added to chicken nugget. They observed that the pH values of the nuggets increased with increasing of amaranth flour but decreased during storage. Additionally, they determined that the TBARS increased during the storage and it was lower in meatballs prepared with amaranth than the control. They emphasized that these results are due to phenolic substances in antioxidant properties in amaranth flour. In another study, 1, 3 and 5% aloe vera powder were added to beef burgers and stored in cold storage for seven days. At the end of storage, it was determined that the lowest pH value was found in samples containing 5% aleo vera and this result was attributed to the acidic character of this plant. The researchers found that pH values decreased during storage and were below 5 in all samples. In addition, they were stated that the amount of TBARS in these meatballs which was initially in the range of 0.52-0.55 mg/kg increased during storage. Nevertheless, the results in samples containing 3% and 5% aleo vera powder were lower than other. They indicated that the low values in these samples were due to phenolic substances in the Aleo vera (Soltanizadeh and Ghiasi-Esfahani, 2015). Park et al. (2016) produced the pork patties with 1% and 3% of buckwheat powder and fermented buckwheat powder and stored at 4 °C for 14 days. Although TBARS values increased during storage, they found that the results in samples containing buckwheat and fermented buckwheat were lower than others. They stated that the lowest value was in the group containing fermented buckwheat as 0.283 mg/kg and the decrease was due to phenolic substances and flavonoids formed in fermentation. In general, our results were similar to these studies and also did not exceed consumption limits reported as pH=6.5 and TBARS=0.7-1 mg malonaldehyde/kg samples by Gökalp et al. (1999). Park et al. (2016) determined that the brightness (L^*) and yellowness (b^*) values of uncooked pork patties decreased with buckwheat flour increasing. They attributed that the decrease in results to the colour substances in buckwheat structure. Park et al. (2017) determined that when salami prepared with buckwheat flour, L^* values increased and b^* values shown fluctuation whereas a values did not change. In addition, they found that L^* values reduced during cold storage and this decline is due to oxidation. In another study, Kilincceker and Yilmaz (2019) found that colours of raw chicken meatballs with different fibre may change according to fibre type and amount. Also, they said that some colour criteria may decreased owing to oxidation in cold storage. The colour results in our study were similar to those mentioned.

4. Conclusions

In this study, the addition of buckwheat flour can affect the quality of chicken meatballs is determined. As the amount of this flour in meatball composition increased, yield and L^* values of fried samples increased whereas diameter reductions, a^* and b^* values decreased. Furthermore, moisture retention ratios during frying increased while oil absorption ratios decreased. The pH and colour characteristics of the stored raw samples showed

decrease in some treatments at the end of the storage, while the amount of TBARS increased. However, pHs increased in samples containing high levels of buckwheat flour on the last storage day, whereas TBARS decreased in the same period and the same samples. It was also determined that the pH and TBARS numbers did not exceed the consumption limit during storage. Consequently, it was understood that buckwheat flour could be mixed with wheat flour and used in the production of chicken meatballs, and the best treatments were 1:3 B:W and 3:1 B:W.

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