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STUDY THE HEAT TRANSFER MODEL IN THE FREEZING PROCESS OF BASA SAUSAGE IN VIETNAM TO DETERMINE THE TECHNOLOGICAL MODE

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ABSTRACT
Based on the results obtained, the mathematical model of heat transfer for freezing food about the relationship between rate of freezing water and freezing temperature of Basa sausage was established ($\omega = f(T)$). Mathematical model of freezing process was solved by variables separable method proposed by Fourier to determine the relationship between rate of freezing water and freezing temperature of Basa sausage, and technological mode of freezing process for preserving Basa sausage. The results showed that when Basa sausage was frozen by air freezing method with the environment temperature of -45°C, the required time for the freezing of water above 86% ($\omega \geq 0.86$) inside Basa sausage was 1.4h. At this point, the temperature of Basa sausage was -24.78°C.

1. Introduction

Application of the technological mode of the freezing process for processing and preservation of food has attracted considerable attention in developed agriculture, fisheries and food countries. The problem posed here is how to determine the optimum freezing temperature of food and freezing time to save energy for the freezing process. Currently, there are two methods to solve that problem:

Firstly, the freezing process reduces the product temperature below the crystallization point from the surface into the center of the product. In this case, the problem is to find the freezing time at which the temperature of the product center reach the crystallization temperature of water inside the product. It means that water inside the product completely crystallized. However, the fact that when the temperature of the product center reach the crystallization temperature, water has not crystallized yet. The cause of this phenomenon is that water inside the product always contents an amount of dissolving compounds. The concentration of the compounds is hard to be examined and changed during the freezing process. Therefore, this method can not find the proper answer.

Secondly, the rate of freezing water inside the product is determined to find the optimum freezing temperature of the product. When water is completely frozen ($\omega = 1$ or 100%), the product temperature is the optimum freezing temperature (Dzung 2007, 2012a & b, 2014).

There are several methods to set up the mathematical model of the freezing process of food. Most of these methods are based on the model of heat transfer and freezing process of food which are built from modeling of complex physical object. Generally, heat transfer model of flat, cylindrical and spherical – shaped

products is properly solved by variables separable method which proposed by Fourier. By contrast, it is hard to clearly solve heat transfer model of complex-shaped products by Fourier methods but digital methods (finite-difference method, finite element method, finite volume method) to find approximate answer.

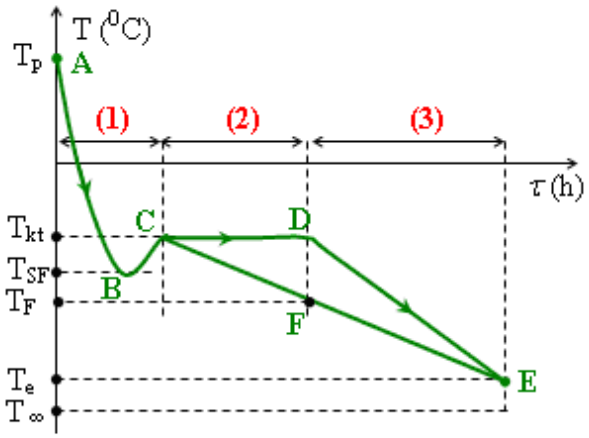
In addition, solving the heat transfer model of complex-shaped products requires the initial hypothesis and initial and boundary conditions. However, water in food always contents dissolving compounds. Therefore, crystallization temperature, latent heat of freezing of water and other thermophysical parameters constantly change during the freezing process. These are the main causes of error between the mathematical models with experimental data. In case of large error, it will not allow the use of mathematical modeling to determine the technological mode. According to overview of Charm et al., (1962), Clary et al., (1968), Cleland, et al., (1976, 1977 & 1979), D. R. Hledman, (1982, 1983 & 1992), Dzung (2007, 2012a & b, 2014), there were many research on mathematical modeling about the freezing time of flat – shaped cattle meat (Plank R et al, 1913), frozen velocity of water inside flat – shaped fish fillet (Lame, Clapeiron, Shijov G.B, 1931), rate of freezing water in wet materials (Plank, Veinik, 1937; Raoult, 1958; Sbijov G.B, 1967; Golovkin N.A, 1972; Luikov, A.V, 1975; D. R. Hledman,1999). However, mathematical modelings of these authors were not suitable for determining rate of freezing water in Basa sausage in Vietnam because experimental results showed that error between the mathematical model and experimental data was higher than 25.39% (Dzung 2007, 2012a & b, 2014).

Therefore, the aim of this study was to build and solve mathematical model of freezing process of Basa sausage. The results obtained were used to establish technological mode of freezing process for preserving Basa sausage (Dzung, 2012a & b).

2.Mathematical model of rate of freezing water

2.1. Description of freezing process

The freezing process of Basa sausage has three stages (Fig.1), (Dzung 2007, 2012a & b, 2014):



AB: cooling stage; BC: extreme cold stage; CD: crystalline water inside materials stage of theory; CF: crystalline water inside materials stage of actuality; DE, FE: super freezing stage.

Figure 1. Basa sausage frozen process

In figure 1, if the process carried out from A to E (A → B → C → F → E), it would be called the cooling and the freezing process. Whereas, if the carried out from E to A (E → F → C → B → A), it would be called the melting and the warm up process (Haugvalstad, et al., 2005).

a) Cooling stage: reduce temperature of Basa sausage (the initial temperature of Basa sausage was $T_P = T_A = 25^0C$ (room temperature)). At the end of this stage, surface and center temperature of Basa sausage were $T_{kt} = -1.08^0C$ and $T_o = 4.27^0C$ respectively.

b) Freezing stage: crystallizing water inside the Basa sausage in freezing environment with temperatures of $T_e = -45^oC$. In this stage, water on the surface area is crystallized first. Consequently, water of successional area is crystallized. This stage finished when the water inside the Basa sausage completely crystallized. At this point,

temperature of Basa sausage is the optimum freezing temperature of T_F (^oC).

c) Energy balance stage: reducing the temperature of Basa sausage from optimum freezing temperature to the final temperature T_e (^oC). This stage was done in a freezing storage room to maintain the temperature lower than T_e (^oC) with $T_e \leq T_F$ (Holman J., 1992).

It is obvious that Basa sausage is frozen to reach the freezing temperature of T_E (^0C), in figure 1, after heat supply to carry out the melting and the warm up process to determine the rate of freezing water in Basa sausage (Gebhart B., 1992; Dzung 2012a & 2014).

The considerable interest here was to determine the relationship between the rate of freezing water and freezing temperature of Basa sausage ($\omega = f(T)$). The results obtained were applied to determine the optimum freezing temperature of Basa sausage ($T = T_{opt}$) in order to completely crystallize water ($\omega = 1$).

2.2. Hypothesis of the establishment of the mathematical model

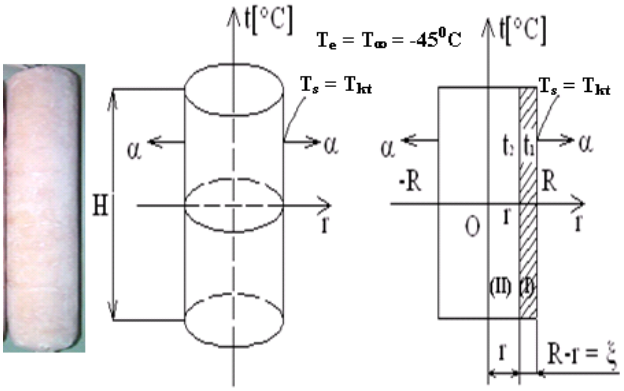


Figure 2. Physical model of Basa sausage

- Basa sausage shape was considered approximately to the infinite cylindrical shape with a diameter of D = 2R, a height of H >>2R. The isothermal surface was concentric cylindrical surface.

- Water was considered homogeneously distributing in Basa sausage. Average value of Latent heat of water was determined with temperature (Dzung 2007, 2012a & b, 2014).

- Average value of thermophysical properties such as: $\rho_i, c_{pi}, a_i, \lambda_i, ...$ were determined with temperature (Gebhart B., 1992; Dzung 2007, 2012a & b, 2014).

- Heat transfer coefficient to the surrounding is constant: $\alpha = const$

- Equation of energy balance between area (I) and area (II) follow the rules of Leibenzon LS (Dzung, 2012a & b, 2014).

2.3. Building and solving of mathematical model of freezing process

From the hypothesis in Section 2.2, equation of heat transfer was written as follow (Gebhart B., 1992; Dzung 2007, 2012a & b, 2014):

$$\frac{\partial t}{\partial \tau} + \vec{w}.gradt = \frac{q_v}{c_p \rho} + a.\nabla^2 t \qquad (1)$$

Basa sausage has infinite cylindrical shape (R<<H). In addition, there are no energy source inside Basa sausage ($q_v = 0$). Because of heat transfer in solid, $\vec{w} = 0$. Besides, the isothermal surface is considered as concentric cylindrical surface. Therefore, the equation (1) is substituted by equation (2) as follow (Dzung 2007, 2012a & b, 2014):

$$\frac{\partial t(r,\tau)}{\partial \tau} = a\left(\frac{\partial^2 t(r,\tau)}{\partial r^2} + \frac{1}{r}\frac{\partial t(r,\tau)}{\partial r}\right) \qquad (2)$$

- In the first area, water inside Basa sausage has crystallized (Dzung 2007, 2012a & b, 2014):

$$\begin{cases} \dfrac{\partial t_1(r,\tau)}{\partial \tau} = a_1\left(\dfrac{\partial^2 t_1(r,\tau)}{\partial r^2} + \dfrac{1}{r}\dfrac{\partial t_1(r,\tau)}{\partial r}\right) \\ r \leq r \leq R\ ,\ \tau \geq 0 \end{cases} \qquad (3)$$

- In the second area, water inside Basa sausage has not crystallized yet (Dzung 2007, 2012a & b, 2014):

$$\begin{cases} \dfrac{\partial t_2(r,\tau)}{\partial \tau} = a_2\left(\dfrac{\partial^2 t_2(r,\tau)}{\partial r^2} + \dfrac{1}{r}\dfrac{\partial t_2(r,\tau)}{\partial r}\right) \\ 0 \le r \le r, \tau \ge 0 \end{cases} \quad (4)$$

- Initial and boundary conditions to solve equation (3) and (4), (Dzung 2007, 2012a & b, 2014):

a) Initial conditions:

When: $\tau = 0$, $T_s = t_1(R,0) = T_{kt}$ (5)

$$T_c = t_2(0,0) = T_0 \quad (6)$$

$$T_e = T_\infty = -45^0 C \quad (7)$$

b) Boundary conditions:

$$\left.\frac{\partial t_1(r,\tau)}{\partial r}\right|_{r=R} = \frac{\alpha}{\lambda_1}\left(t_1(R,\tau) - T_e\right) \quad (8)$$

$$\left.\frac{\partial t_2(r,\tau)}{\partial r}\right|_{r=0} = 0 \quad (9)$$

c) In the contact surface of first (I) and second (II) areas:

$$t_1(r,\tau) = t_2(r,\tau) = T_{kt} \quad (10)$$

- Equation of energy balance in the contact surface of area (I) and area (II):

$$\begin{aligned} -Ld(GW_0\omega) &= -L\rho_1\pi R^2 H W_0 d\omega \\ &= -\lambda_1\left(\frac{\partial t_1}{\partial r}\right)_{r=r^-} 2\pi r H d\tau + \lambda_2\left(\frac{\partial t_2}{\partial r}\right)_{r=r^+} 2\pi r H d\tau \end{aligned} \quad (11)$$

Therefore:

$$\begin{aligned} &\omega(r,\tau) \\ &= \frac{1}{L\rho_1 R^2 W_0}\int_0^\tau\left(\left[\lambda_1\left(\frac{\partial t_1}{\partial r}\right)_{r=r^-} - \lambda_2\left(\frac{\partial t_2}{\partial r}\right)_{r=r^+}\right]2r\right)d\tau \end{aligned} \quad (12)$$

Where: λ_1, λ_2 ($W.m^{-1}.K^{-1}$): heat transfer coefficient of area (I) and area (II); ρ_1 ($kg.m^{-3}$): density of area (I); ω: rate of freezing water; W_0: initial moisture of Basa sausage; L ($J.kg^{-1}$): latent heat of freezing of water; a_1, a_2 ($m^2.s^{-1}$): thermal diffusivity of area (I) and area (II).

- **Solving equation (3):** using Fourier separable method. With: $t_1(r,\tau) = \psi_1(r)\varphi_1(\tau)$, constants of integration were determined from initial and boundary conditions. Through some transformations, the roots obtained were presented as follow (Gebhart B., 1992; Dzung 2007, 2012a & b, 2014):

$$\begin{aligned} &t_1(r,\tau) = T_\infty + \\ &(T_{kt} - T_\infty)\sum_{n=1}^{\infty} 2\frac{J_1(\mu_n)}{\mu_n[J_0^2(\mu_n) + J_1^2(\mu_n)]} J_0(\mu_n \frac{r}{R}) \exp(-\mu_n^2 Fo_1) \end{aligned} \quad (13)$$

$$\begin{aligned} &\left.\frac{\partial t_1}{\partial r}\right|_{r=r^-} = \\ &-\frac{(T_{kt} - T_\infty)}{R}\sum_{n=1}^{\infty} 2\frac{J_1(\mu_n)}{[J_0^2(\mu_n) + J_1^2(\mu_n)]} J_1(\mu_n \frac{r}{R}) \exp(-\mu_n^2 Fo_1) \end{aligned} \quad (14)$$

Where: $T_\infty = -45^0 C$: temperature of freezing environment; $r \le r \le R$, $\tau \ge 0$

T_{kt} = -1.08^{0}C: freezing temperature of water inside Basa sausage

μ_n : Root of specific equation:

$$\frac{J_0(\mu_n)}{J_1(\mu_n)} = \frac{\mu_n}{Bi_1} \quad (15)$$

Bi_1: Biot number of area (I):

$$Bi_1 = \alpha R / \lambda_1 \quad (16)$$

Fo_1: Fourier number of area (I):

$$Fo_1 = a_1\tau / R^2 \quad (17)$$

$J_0(\mu_n)$, $J_1(\mu_n)$: Bessel functions type 1 of the zero and first order**.**

- **Solving equation (4):** similar to solving equation (3). With: $t_2(r,\tau) = \psi_2(r)\varphi_2(\tau)$, constants of integration were determined from initial and boundary conditions. The root obtained was as follow (Gebhart B., 1992):

$$\begin{aligned} &t_2(r,\tau) = T_{kt} + \\ &(T_0 - T_{kt})\sum_{m=1}^{\infty} 2\frac{J_1(\mu_m)}{\mu_m[J_0^2(\mu_m) + J_1^2(\mu_m)]} J_0(\mu_m \frac{r}{R}) \exp(-\mu_m^2 Fo_2) \end{aligned} \quad (18)$$

$$\begin{aligned} &\left.\frac{\partial t_2}{\partial r}\right|_{r=r^+} = \\ &-\frac{(T_0 - T_{kt})}{R}\sum_{m=1}^{\infty} 2\frac{J_1(\mu_m)}{[J_0^2(\mu_m) + J_1^2(\mu_m)]} J_1(\mu_m \frac{r}{R}) \exp(-\mu_m^2 Fo_2) \end{aligned} \quad (19)$$

Where: T_0 = 4.27^{0}C: temperature of Basa sausage center at $\tau = 0$ và $T_s = T_{kt}$

$T_{kt} = -1.08^0 C$: freezing temperature of water inside Basa sausage $0 \le r \le r, \tau \ge 0$

μ_m: Root of specific equation:

$$\frac{J_0(\mu_m)}{J_1(\mu_m)} = \frac{\mu_m}{Bi_2} \quad (20)$$

Bi_2: Biot number of area II:

$$Bi_2 = \alpha R / \lambda_2 \quad (21)$$

Fo_2: Fourier number of area II:

$$Fo_2 = a_2 \tau / R^2 \quad (22)$$

$J_0(\mu_m)$, $J_1(\mu_m)$: Bessel functions type 1 of the zero and first order.

$$J_0(x) = 1 - (\frac{1}{2}x)^2 + \frac{(\frac{1}{2}x)^4}{1^2.2^2} - \frac{(\frac{1}{2}x)^6}{1^2.2^2.3^2} + \frac{(\frac{1}{2}x)^8}{1^2.2^2.3^2.4^2} - ... \quad (23)$$

$$J_1(x) = -J_0'(x) = \frac{1}{2}x - \frac{(\frac{1}{2}x)^3}{1^2.2} + \frac{(\frac{1}{2}x)^5}{1^2.2^2.3} - \frac{(\frac{1}{2}x)^7}{1^2.2^2.3^2.4} + ... \quad (24)$$

- **Mathematical model for determining rate of freezing water**

Approximate form of Bessel function:

$$\begin{cases} J_1(\mu_n \frac{r}{R}) \approx \mu_n \frac{r}{2R}; & 0 \le \left|\mu_n \frac{r}{2R}\right| << 1 \\ J_1(\mu_m \frac{r}{R}) \approx \mu_m \frac{r}{2R}; & 0 \le \left|\mu_m \frac{r}{2R}\right| << 1 \end{cases} \quad (25)$$

Substituting (14), (19) into (21) and (12) will obtained equation of rate of freezing water as follow (Dzung 2007, 2012a & b, 2014):

$$\omega(r,\tau) = \frac{2r^2}{L\rho_1 W_0 R^2}\left[-c_1\rho_1(T_{kt} - T_\infty)\sum_{n=1}^{\infty}\frac{J_1(\mu_n)}{[J_0^2(\mu_n) + J_1^2(\mu_n)]}\left[1 - \exp(-\mu_n^2 Fo_1)\right] + c_2\rho_2(T_0 - T_{kt})\sum_{m=1}^{\infty}\frac{J_1(\mu_m)}{[J_0^2(\mu_m) + J_1^2(\mu_m)]}\left[1 - \exp(-\mu_m^2 Fo_2)\right]\right] \quad (26)$$

The average value of rate of freezing water with temperature was determined as follow:

$$\bar{\omega}(\tau) = \frac{1}{V}\iiint_V \omega(r,\tau)dV = -\frac{c_1(T_{kt} - T_\infty)}{LW_0}\sum_{n=1}^{\infty}\frac{J_1(\mu_n)}{J_0^2(\mu_n) + J_1^2(\mu_n)}\left(1 - \exp(-\mu_n^2 Fo_1)\right) + \frac{c_2\rho_2(T_0 - T_{kt})}{L\rho_1 W_0}\sum_{m=1}^{\infty}\frac{J_1(\mu_m)}{J_0^2(\mu_m) + J_1^2(\mu_m)}\left(1 - \exp(-\mu_m^2 Fo_2)\right) \quad (27)$$

With: $A_n = \frac{J_1(\mu_n)}{J_0^2(\mu_n) + J_1^2(\mu_n)}$;

$A_m = \frac{J_1(\mu_m)}{J_0^2(\mu_m) + J_1^2(\mu_m)}$;

$B_1 = \frac{c_1(T_{kt} - T_\infty)}{LW_0}$; $B_2 = \frac{c_1(T_{kt} - T_\infty)}{LW_0}$

Therefore, mathematical model (27) was rewritten as follow:

$$\bar{\omega}(\tau) = -B_1\sum_{n=1}^{\infty}A_n\left(1 - \exp(-\mu_n^2 Fo_1)\right) + B_2\sum_{m=1}^{\infty}A_m\left(1 - \exp(-\mu_m^2 Fo_2)\right) \quad (28)$$

Average temperature of Basa sausage was determined via the following equation:

$$T = \frac{1}{2R}\frac{1}{\tau}\int_0^{\tau}\left(\int_0^R t_1(r,\tau)dr + \int_0^R t_2(r,\tau)dr\right)d\tau \quad (29)$$

2.3. Materials and methods

2.3.1. Materials

Basa fish of approximately (1.2 ÷ 1.5) kg/a fish grown in the DBSCL of Vietnam was cut off head, viscera and ground into small pieces to make sausage.

2.3.2. Apparatus

Equipments used to determine specific heat of Basa sausage are listed:

Determining weigh of Basa sausage by Satoriusbasic Type BA310S Weigh: range scale (0 ÷ 350)g, error of equipment: ± 0.1g = ± 0.0001 kg.

Determining temperature of Basa sausage by Thermometer (Dual Digital Thermometer):

range scale (-50 ÷ 70)^{0}C, error of equipment ± 0.05^{0}C.

DL-3 freezing System (Fig. 3) could reduce the temperature of environment to (-50 ÷ -45)^{o}C. The temperature profile was measured by the automatic control system PLC.

Equipment used to identify specific heat was shown in Fig.3. The equipment includes a Voltmeter (range scale: (0 ÷ 110)V, error: ± 1V), an amperemeter (range scale: (0 ÷ 2)A, error: ± 10mA) and an automatic timer (error: ± 0.001s). The Voltmeter was used to measure the potential difference of resistance (R). The Amperemeter was used to determine the current intensity which passes through 2 resistances (R) (Fig 4).

Fig 3. The Refrigeration system DL-3 with the auto freezing (-45 ÷ - 40)^{0}C

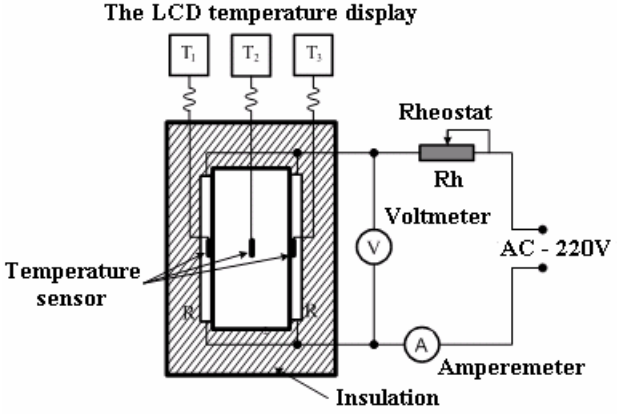


Fig 4. Principle diagram of the equipment determine the specific heat of moist material

2.3.3. Methods

Determing rate of freezing water by experimental method (Dzung and et al, 2012a & b, 2014)

Rate of freezing water ω $(0 \le \omega \le 1)$ was determined via heat supply to carry out the melting and the warm up process to determine the rate of freezing water in Basa sausage. According to Dzung N.T and et al., (2012a, 2014), Rate of freezing water inside Basa sausage was determined as follow:

$$\omega = \frac{(1-\varphi)UI\tau - \phi_1 - \phi_2(T_c - T_d)}{\phi_3(T_c - T_d) - \phi_1} \qquad (29)$$

Where: T (^{0}C) – average temperature of Basa sausage, $T = (T_d + T_c)/2$,

$\varphi = 0.1101$ is the loss of heat coefficient,

With: $\phi_1 = LW_aG$;

$\phi_2 = (c_nW_a + c_{ck}(1\text{-}W_a))G + c_1G_1$;

$\phi_3 = GW_a(c_{nd} - c_n)$

Where: c_1(J.kg^{-1}.K^{-1}), G_1 (kg): specific heat and weight of flat-shaped copper in the equipment in Fig.4; G (kg) weight of Basa sausage sample; $T_d = T_1 = T_2 = T_3$ (^{0}C): temperature of Basa sausage sample without energy supply; $T_c = T_1' = T_2' = T_3'$ (^{0}C): temperature of Basa fillet sample after supplying energy; U(V): number of voltmeter, I(A): number of amperermeter, τ(s): energy supply time.

The equipment (Fig 4) was surrounded by heat-insulated material to ensure that energy from electric resistance only transmit to Basa sausage sample. There was no loss to surrounding.

Mathematical tools were use to solve the mathematical model about the relationship between rate of freezing water and freezing temperature of Basa sausage and evaluate error between mathematical model and experimental data.

3. Results and discussions

3.1. Results

The thermophysical parameters of Basa sausage were used to solve mathematical model of freezing process and to determine the rate of freezing water (Dzung et al, 2012a & b, 2014). These parameters were presented in Table 1.

Table 1. Thermophysical parameters of Basa sausage

	Value	Unit	References		Value	Unit	References
W_0	73.82	%	Dzung 2012b	ρ_2	927.32	$Kg.m^{-3}$	Dzung 2007
R	$8.0x10^{-3}$	m	Dzung 2007	λ_1	1.104	$W.m^{-1}.K^{-1}$	Dzung 2007
h	$20x10^{-2}$	m	Dzung 2007	λ_2	0.524	$W.m^{-1}.K^{-1}$	Dzung 2007
T_{kt}	-1.08	^0C	Dzung 2012b	c_{ck}	1661.31	$J.kg^{-1}.K^{-1}$	Dzung 2007
T_0	4.27	^0C	Dzung 2007	c_1	2613.21	$J.kg^{-1}.K^{-1}$	Dzung 2007
T_∞	-45	^0C	Dzung 2012b	c_2	3498.23	$J.kg^{-1}.K^{-1}$	Dzung 2007
L	333600	$J.kg^{-1}$	Perry and et al. 1992	a_1	$4.629x10^{-7}$	$m^2.s^{-1}$	Calculation
ρ_1	912.56	$Kg.m^{-3}$	Dzung 2007	a_2	$1.615x10^{-7}$	$m^2.s^{-1}$	Calculation

3.1.1. Determining heat transfer coefficient

Surface temperature $t_1(R, \tau)$ and center temperature $t_2(0, \tau)$ of Basa sausage was determined via solving equation (15) and (20) to find values of μ_n, μ_m. Therefore, heat transfer coefficient of air freezing environment at -45°C (α, $W.m^{-2}.K^{-1}$), Bi_1 and Bi_2 were determined before solving equation (15) and (20).

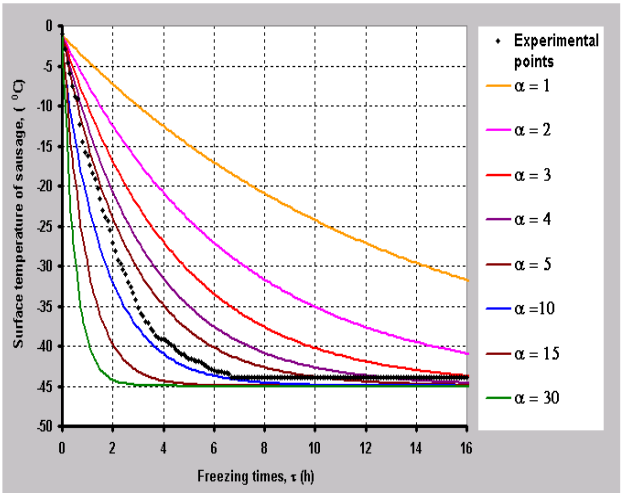


Figure 5. Surface temperature of Basa sausage

Determining heat transfer coefficient via the optimization problem was conducted through 4 steps (Dzung et al., 2012a & b, 2014):

- **Step 1**: $\alpha = \alpha_j$ varied from 1 to 30. When $\Delta\alpha_j$ was equal to 1 ($\Delta\alpha_j = 1$), α_j was substituted into equation (16) and (21) to determine Bi_1 and Bi_2. Solving equation (15) and (20) would be obtained value of μ_n, μ_m. Subsequently, μ_n, μ_m were substituted into equation (13) and (18) to determine $t_1(R, \tau)$, $t_2(0, \tau)$. With each value of α_j, $t_1(R, \tau)\big|_{\alpha_j}$ and $t_2(0, \tau)\big|_{\alpha_j}$ were determined. A Matlab 7.0 software was used to calculate $t_1(R, \tau)\big|_{\alpha_j}$ and $t_2(0, \tau)\big|_{\alpha_j}$. The results were presented in Fig.5 and Fig. 6.

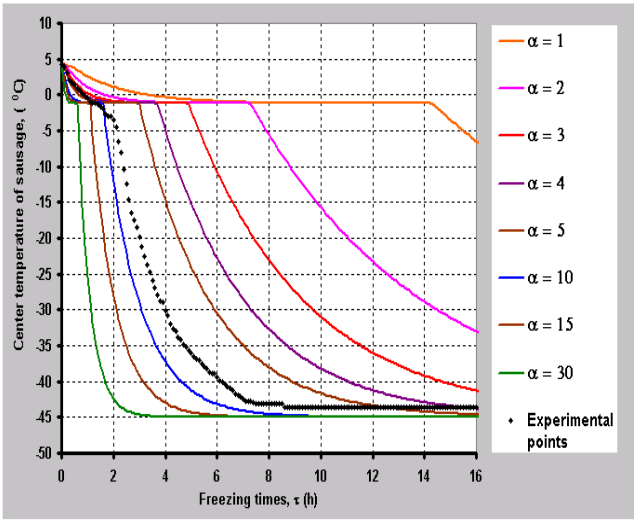


Figure 6. Center temperature of Basa sausage

- **Step 2**: center temperature ($T_{cE}(\tau)$, ^0C) and surface temperature ($T_{sE}(\tau)$, ^0C) of Basa sausage with freezing time were determined by experiments. The results were shown in Fig.5 and Fig. 6. It can be clearly seen from Fig.5 and Fig.6 that curves of $T_{cE}(\tau)$ and $T_{sE}(\tau)$ were interjacent between curves of $t_2(0, \tau)$, $t_1(R, \tau)$ when $\alpha_j = 5$ and $\alpha_j = 10$.

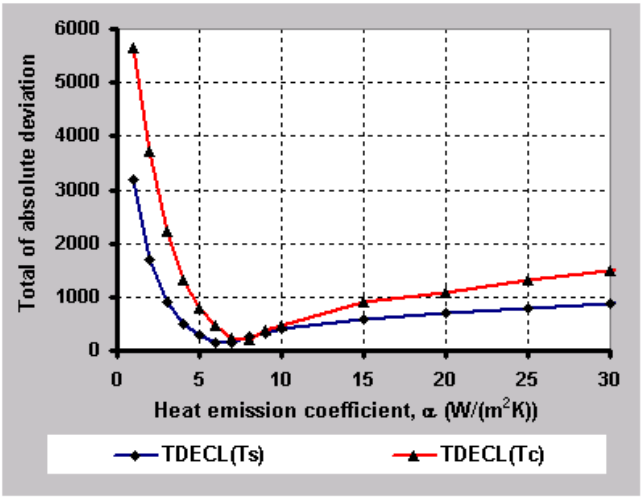


Figure 7. Total of absolute deviation, TAD(Ts) and TAD(Tc)

- **Step 3**: α_j was varied from 5 to 10 and $\Delta\alpha_j = 0.001$. Similar to step 1 and step 2, with each value of α_j, $t_1(R, \tau)\big|_{\alpha j}$ và $t_2(0, \tau)\big|_{\alpha j}$ were determined. The optimization problem was to find total of absolute deviation (TAD) between $T_{sE}(\tau)$ and $t_1(R, \tau)\big|_{\alpha j}$, between $T_{cE}(\tau)$ and $t_2(0, \tau)\big|_{\alpha j}$. Optimum heat transfer coefficient of freezing environment was determined when the TAD reached the minimum value.

$$TAD(T_s) = \sum_{i=1}^{N} \left| T_{sE}(\tau) - t_1(R,\tau) \right|_{\alpha j} \rightarrow \min \qquad (30)$$

$$TAD(T_c) = \sum_{i=1}^{N} \left| T_{cE}(\tau) - t_2(0,\tau) \right|_{\alpha j} \rightarrow \min \qquad (31)$$

The Matlab 7.0 software was used and the results were shown in Fig.7. When $\alpha_j = 7.982$ $W.m^{-2}.K^{-1}$, value of $TAD(T_s)_{min}$ and $TAD(T_c)_{min}$ were minimum ($TAD(T_s)_{min}$ = 154.78 and $TAD(T_c)_{min}$ = 209.29).

- **Step 4:** checking the compatibility of the mathematical models (13) and (18) with $\alpha = \alpha_j = 7.982$ $W.m^{-2}.K^{-1}$. The α of 7.982 $W.m^{-2}.K^{-1}$ was substituted into equations (16) and (21) to find Bi_1, Bi_2. Equations (15) and (20) were solved to find μ_n, μ_m. Consequently, μ_n, μ_m were substituted into equations (13), (18) to determine $t_1(R, \tau)$, $t_2(0, \tau)$. The error of mathematical (13) and (18) were determined as follow:

$$Error(T_s) = \frac{\sum_{i=1}^{N} \left| T_{sE}(\tau) - t_1(R,\tau) \right|_{\alpha j = 7.982}}{\sum_{i=1}^{N} \left| T_{sE}(\tau) \right|_{\alpha j = 7.982}} = \frac{154.78}{6614.53} = 2.34\% \qquad (32)$$

$$Error(T_c) = \frac{\sum_{i=1}^{N} \left| T_{cE}(\tau) - t_2(0,\tau) \right|_{\alpha j = 7.982}}{\sum_{i=1}^{N} \left| T_{cE}(\tau) \right|_{\alpha j = 7.982}} = \frac{209.29}{7136.975} = 3.57\% \qquad (33)$$

If the error of mathematical models (13) and (18) were chosen lower than 5%, it could be seen clearly that mathematical model (13) and (18) were completely compatible with experimental data because the error of mathematical model (13) and (18) with experimental data were 2.34% and 3.57% respectively. Therefore, these mathematical models could be used to determine rate of freezing water.

From the results obtained, it could be concluded that appropriate heat emission coefficient of air freezing environment at -45°C was $\alpha = 7.982$ $W.m^{-2}.K^{-1}$.

3.1.2. Determining μ_n, μ_m roots of equation(15) and (20), and coefficients A_n, A_m, B_1, B_2

- Substituting $\alpha = 7.982$ $W.m^{-2}.K^{-1}$ into (16) and (21) to find $Bi_1 = 0.0578$; $Bi_2 = 0.1219$ (Dzung, 2012b, 2014)

- Substituting thermophysical parameters in Table 1, Bi_1 and Bi_2 into specific equations (15) and (20); solving the equations to find μ_n, μ_m; choosing value of n, m for value of (13) and (18) quickly converge. The results were shown in Table 2.

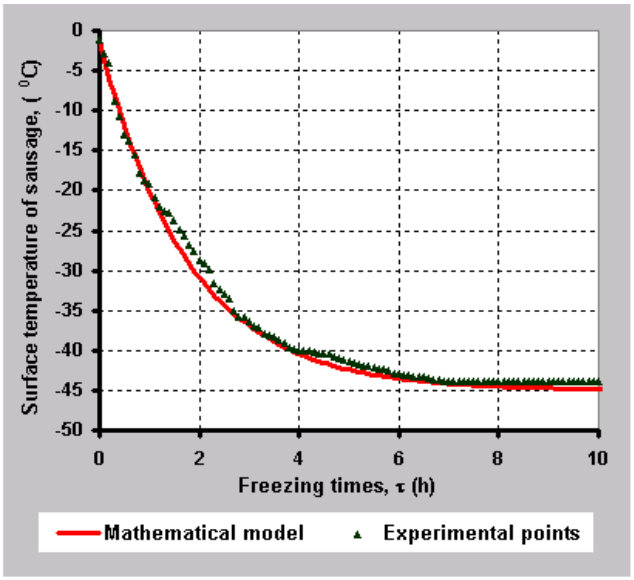


Figure 8a. Relationship between Surface temperature (T_s) and freezing time of Basa sausage

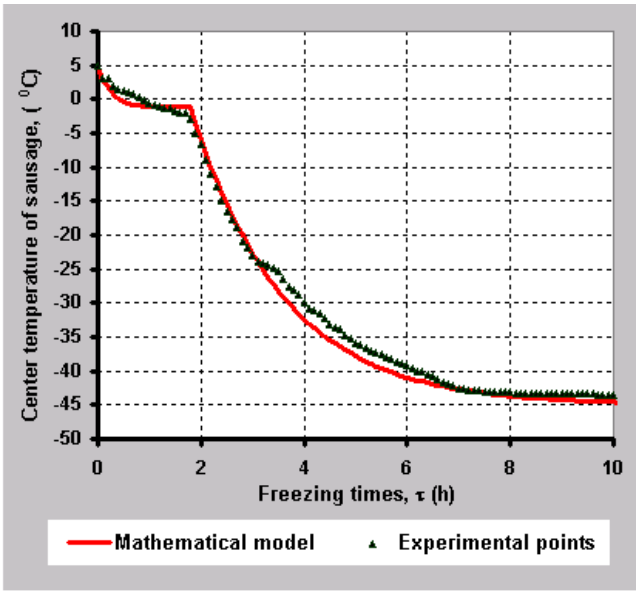


Figure 8b. Relationship between Center temperature (T_c) and freezing time of Basa sausage

Table 2. Roots of equations (15), (20) and values of Bessel function, A_n, A_m, B_1, B_2

n	1	2	3	4	
μ_n	0.3377	3.8468	7.0238	13.328	
$J_0(\mu_n)$	0.9717	-0.4027	0.3001	0.2184	
$J_1(\mu_n)$	0.1665	-0.0061	0.0025	0.0009	
A_n	0.17131	-0.03761	0.0277573	0.018868	
B_1	0.466054				
m	1	2	3	4	5
μ_m	0.4863	3.8634	7.0329	13.3328	16.478
$J_0(\mu_m)$	0.9417	-0.4026	0.3001	0.2184	-0.1965
$J_1(\mu_m)$	0.2360	-0.0127	0.0052	0.0020	-0.00145
A_m	0.250399	-0.07828	0.0577219	0.041926	-0.03755
B_2	0.633985				

3.1.3. Determining surface, center and average temperature, and rate of freezing water of Basa sausage in the freezing process

Surface, center and average temperature, and rate of freezing water of Basa sausage in the air freezing process with environment temperature of -45°C have been found by substituting thermophysical parameters in Table 1, roots of equation (15) and (20), and parameters A_n, A_m, B_1 and B_2 in Table 2 into equations (13), (18), (28) and (29). The results were illustrated in Table 3.

Experiments were also carried out to find rate of freezing water (RWF) with average temperature of Basa sausage using equation (33). The results were also shown in Table 3.

Table 3. Surface, center and average temperature, and rate of freezing water (RFW) of Basa sausage from mathematical model and experiment

τ (h)	$t_1(R, \tau)$	$t_2(0,\tau)$	T	ω_M	ω_E
0	-1.08	4.27	1.33	0.0000	0
0.2	-21.09	1.23	-11.05	0.3507	0.2667
0.4	-31.80	0.42	-17.30	0.5716	0.4583
0.6	-37.71	-0.10	-20.79	0.6974	0.5732
0.8	-40.98	-0.44	-22.74	0.7899	0.6957
1	-42.78	-0.67	-23.83	0.8561	0.7365
1.2	-43.77	-0.81	-24.44	0.9024	0.7768
1.4	**-44.32**	**-0.90**	**-24.78**	**0.9344**	**0.8621**
1.6	-44.63	-0.97	-24.98	0.9562	0.8943
1.8	-44.79	-1.01	-25.09	0.9709	0.9135
2	-44.89	-1.03	-25.15	0.9808	0.9422
2.2	-44.94	-1.05	-25.19	0.9874	0.9514
2.4	-44.97	-1.07	-25.21	0.9918	0.9605
2.6	**-44.98**	**-1.08**	**-25.23**	**1.0000**	0.9713
2.8	-44.99	-1.25	-25.31	1.0000	0.9824
3	**-44.99**	**-16.83**	**-32.32**	**1.0000**	**1.0000**
3.2	-45.00	-26.67	-36.75	1.0000	1.0000
3.4	-45.00	-33.07	-39.63	1.0000	1.0000
3.6	-45.00	-37.24	-41.51	1.0000	1.0000
3.8	-45.00	-39.95	-42.73	1.0000	1.0000
4	-45.00	-41.71	-43.52	1.0000	1.0000

From the results in Table 3, the relationship between rate of freezing water (RFW), surface temperature, center temperature and average temperature of Basa sausage with freezing time were demonstrated in Fig 9.

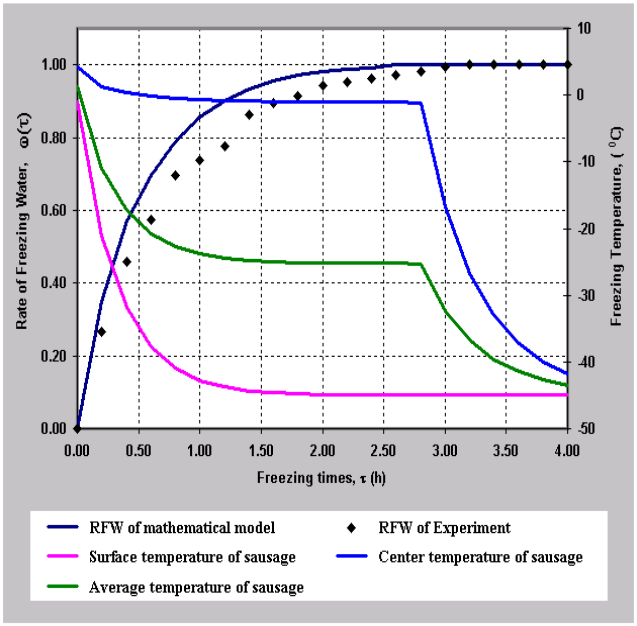


Figure 9. Relationship between rate of freezing water and temperature of Basa sausage with freezing time

3.1.4. Determing error of mathematical model with experimental data

Average error of ω_M from mathematical model and ω_E from experiment was determined as the following equation (Dzung et al., 2007, 2012a & b, 2014):

$$\text{Error}(\omega) = \frac{\left|\int_{T_{i=1}}^{T_{i=n}} \omega_M(\tau)d\tau - \int_{T_{i=1}}^{T_{i=n}} \omega_E(\tau)d\tau\right|}{\int_{T_{i=1}}^{T_{i=n}} \omega_E(\tau)d\tau}100\% = \frac{\sum_{i=1}^{n}\left|\omega_M - \omega_E\right|\Delta\tau}{\sum_{i=1}^{n}\omega_E\Delta\tau}100\% \quad (34)$$

The results obtained in Table 3 were substituted into equation (30) and (31) to determine error of mathematical model (28) with experimental data by equation (34):

$$\text{Error}(\omega) = \frac{\sum_{i=1}^{n}\left|\omega_M - \omega_E\right|}{\sum_{i=1}^{n}\omega_E(T)}100\% = \frac{1.0091}{16.9805}100\% = 5.94\%$$

3.1.5. Determining technological mode of the freezing process of Basa sausage

From the results obtained in Table 3 and Fig 9, technological mode of freezing process of Basa sausage was established and shown in Table 4.

Table 4. Technological mode of the freezing process of Basa sausage

Parameters	Symbol & Unit	Value
Heat emission coefficient	α, $W.m^{-2}.K^{-1}$	7.982
Freezing time	τ, h	1.4
Freezing point of water inside Basa sausage	T_{kt}, ^0C	-1.08
Environmental freezing temperature	T_∞ , ^0C	-45
Average temperature of Basa sausage	T, ^0C	-24.78
Rate of freezing water	ω_E	0.8621
Rate of freezing water	ω_M	0.9344

When Basa sausage was frozen with technological mode as shown in Table 4, the products of the freezing process were presented in Fig 10.

Figure 10. Products of freezing Basa sausage

3.2. Discussion

- Optimum heat emission coefficient α = 7.982 $W.m^{-2}.K^{-1}$ in the freezing environment of -45^{o}C was determined by solving inverse problem of freezing process. Error between mathematical model (13) and (18) with experimental data were 2.34% (<5%) and 3.57% (<5%). It was clearly that when α = 7.982 $W.m^{-2}.K^{-1}$, mathematical models (13) and (18) well described gradient temperature of surface and center of Basa sausage in the freezing process. Therefore, these mathematical models could be used to determine rate of freezing water. However, when freezing method and temperature of freezing environment were changed, value of α would also change.
- It can be clearly seen from Fig 9 that mathematical model (28) well described the relationship between rate of freezing water and freezing time of Basa sausage. When the center temperature reached the crystallization temperature, rate of freezing water from mathematical model (28) was 100%. However, rate of freezing water from experiment was lower than 100%. The main cause of this phenomenon was that crystallization temperature of water inside Basa sausage constantly changed during the freezing process.
- The results showed that error between mathematical model (28) and experimental data was 5.94%. There were four reasons that lead to the error:

- Firstly, thermophysical parameters (ρ, c, λ) of Basa sausage were averaged and considered as constant. However, during the changing phage of water in the freezing process, these parameters constantly changed.
- Latent heat of freezing was not a constant number but constantly changing with the changing of temperature of the freezing process.
- Water inside the Basa sausage contents an amount of dissolving compounds. Therefore, crystallization temperature of water was not a constant number but constantly changing during the freezing process.
- Finally, water dose not homogeneously distribute in Basa sausage. In addition, physical model was just approximate to finite cylindrical shape. Hence, symmetrical conditions were only approximate to theory.

However, error between mathematical model (28) with experimental data was completely acceptable (5.94%). Therefore, the

mathematical model could be used to set up technological mode of the freezing process for Basa sausage preservation.

- Currently, factories often reduce the freezing temperature of Basa sausage to (-35 ÷ -30)^{0}C. In such low temperature, microorganisms test showed that microorganisms could not grow, reproduce and most of the microorganisms are inactivated. However, the optimal freezing temperature has not exactly determined yet. Technological mode of the freezing process for Basa sausage preservation was shown in Table 4. The results were not only completely compatible with large-scale process but also a technological solution for factories to improve the freezing process, saving energy costs when the freezing system is operated.

Nomenclature

$\omega \in [0,1]$: Rate of freezing water

ω_E: Rate of freezing water determined by experimental method

ω_M: Rate of freezing water determined by mathematical model

$W_o = 0.7382 = 73.82\%$ Initial moisture of Basa sausage

$c_n = 4184.7 + 2.74T$ (J.kg^{-1}.K^{-1}) Specific heat of water

$c_{nd} = 2090 + 7.79T$ (J.kg^{-1}.K^{-1}) Specific heat of ice

$c_{ck} = 1739.43 + 1.3155T$ (J.kg^{-1}.K^{-1}) Specific heat of the dry matter inside Basa sausage

c (J.kg^{-1}.K^{-1}) Specific heat of Basa sausage when water is crystallized

$c_1 = 380.21$ (J.kg^{-1}.K^{-1}) Specific heat of copper

G (kg) Weight of Basa sausage sample

$G_1 = 0.025$ (kg) Weight of flat-shaped copper in equipment determine specific heat of moist material.

$T_{kt} = -1.08^0$C Freezing point of water inside Basa sausage

$T_p = 25^0$C Room temperature

T_F (^{0}C) Temperature of Basa sausage when water completely crystallized.

$T_d = T_1 = T_2 = T_3$ (^{0}C) Initial temperature of Basa sausage sample

$T_c = T_1' = T_2' = T_3'$ (^{0}C) Temperature of Basa sausage sample after supplying energy

$T = (T_d + T_c)/2$ (^{0}C) Average temperature of Basa sausage

U (V) Number of voltmeter

I (A) Number of amperemeter

τ (s) Heat supply time

λ_1, λ_2 (W.m^{-1}.K^{-1}) Thermal conductivity coefficient of first (I), second (II) areas

ρ (kg.m^{-3}) Density of first area (I)

L (J.kg^{-1}) Latent heat of freezing of water

a_1, a_2 (m^2.s^{-1}) Thermal diffusivity of first (I) and second (II) areas

Bi_1, Bi_2: Biot number of areas (I) and (II)

Fo_1, Fo_2: Fourier number of areas (I) and (II)

4. Conclusions

- The study has determined optimal heat emission coefficient (α = 7.982 W.m^{-2}.K^{-1}) of freezing environment at -45^{o}C. Mathematical model (13) and (18) were completely compatible with experimental data and could be used to determine rate of freezing water.
- The study has also built mathematical model (28). The mathematical model well described relationship of rate of freezing water with freezing time of Basa sausage. In addition, error of mathematical model with experimental data was 5.94% and the mathematical model (28) could be used to calculate and set up technological mode of the freezing process for preserving Basa sausage.
- Technological mode in Table 4 was established from mathematical model (28) and the results were completely compatible with practical process for preserving Basa sausage.

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OPTIMIZATION OF FREEZING CHERRY FRUITS BY VARIOUS PRE-TREATMENT METHODS

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ABSTRACT

Showing preprocessing cherry fruit of varieties Shpanka and Lotovka by sugar solution with the addition of chitosan or ascorutin. For this cherry fruit kept in 20% sugar solution with the addition of 4% ascorutin or 20% sugar solution with the addition of 1% chitosan for 30 minutes, dried by blowing air and frozen by crumbly at −25ºC, packed in plastic bags for up 0.5 kg and stored at –18 °C. By taking for control nontreated of cherry fruit and packed in plastic bags. According to the research, preservation of quality and biological value of frozen fruit cherry preprocessing contributes 20% sugar solution with the addition of 1% chitosan. Thus their antioxidant activity is 27 and 18 mmol/dm^3 ascorbic acid content of 17.6 and 20 mg/100g. On the basis of the conducted correlational and regressive analysis is obtained a mathematical model of definition antioxidant activity of fruit cherries.

1.Introduction

Cherry (Cerasus prunus L.) is one of the most attractive fruit in the food industry due to it nutritive value and organoleptic properties. It is characterized by intense red color and sweet-sour taste with high content of bioactive components, polyphenols and organic acids, which are beneficial for human health (Tarhan et al., 2006). Anthocyanins, which cause red cherry fruit, is exhibit antioxidant, anti-inflammatory, antibacterial, antidiabetic properties (Halvorsen et al., 2002). Organic acids stimulate the activity of digestive enzymes (Seymour et al., 2008). Also fruit cherries contain all the important vitamins: C − 20 mg/100g and B_1 – 50 mg/100g, B_2− 20mg/100g and others (Steger-Matea et al., 2010).

However cherries – a seasonal product, the term of harvest which only one month, and most of the technologies of causing significant deterioration of biologically active compounds. Therefore, in order to minimize the loss of raw materials is need to develop methods for pre-processing, which can keep the nutritional value without causing an increase in losses in the process (Nowicka et al., 2015).

One of these methods is the freezing by osmotic dehydration of raw materials, using a solution of sucrose, glucose, fructose (Nowicka et al., 2015; Jissy and Gopinadhan, 2012; Zhao et al., 2014; Bchir et al., 2012; Korobkina et al., 1979).

Also, for reduce the loss of anthocyanins and therefore antioxidant activity, use paths of enriching and adding antioxidants in foods. It is

possible to extend storage due to oxidation of lipids, which are a cause of deterioration in food quality (Yeremeeva and Makarova, 2015). One of these antioxidants Shaheen M.S. suggested the use of chitosan-fructose to prevent lipid oxidation in meat (Mohamed et al., 2016). Also according to Jiang et al. (2012) solution containing 3% chitosan, 0.75% glycerol, 0.25% Sapn, 40.5% corn syrup, 3% pectin solution, 88% distilled water was used to pre-freeze of blueberries. This reduces the loss of density, juice and improves its quality.

The value of preparation as ascorutin is that its ingredients are ascorbic acid and rutin − biologically active substances, that potentiate the effect of each other, resulting in inhibition of peroxidation oxidation, thus preserving the high quality of the product (Kalytka et al., 2006).

Antioxidant fruit properties are characterized by vitamin C, polyphenols and anthocyanins content. Among these compounds, there is correlation dependence, which recently used to create functional of wholefood (Stager-Mate et al., 2010, Poiana et al., 2012).

Recently, studies Almeida (2005), Quintereo-Ramos (1998) and Sin (2006) et al., shows osmotic dehydration process optimization and its influence on the transmission processes of drying and freezing. Particularly in the Almeida (2005) shows the optimization process of osmotic dehydration and drying of mango and established concentration of 40% sucrose, 90 minutes immersion time and temperature drying 50°C.

In work of Sumić et al. (2015) been optimized drying process with the development of desirable features for maximum content of total phenols and vitamin C, anthocyanins in dried blueberries with a minimum color change.

As the cherry fruit is rich in tanning and coloring substances and ascorbic acid, which have antioxidant properties and given that after thawing the fruit content of these substances varies considerably. We set the goal to optimize the process of freezing fruits cherries and examine the contents of tannins and coloring matter, vitamin C, antioxidant and water-retaining capacity of frozen fruit cherry pretreated with a sugar solution with added the substances of antioxidant activity − ascorutin and chitosan.

2. Materials and methods

Studies conducted over the years 2015−2016 with the fruits of cherry varieties Shpanka and Lotovka. Prepare fruit included: sorting, inspection, washing, hold 30 minutes in solutions of 20% sugar ascorutin 4% or 20% of the sugar with the addition of 1% chitosan, remove moisture, freezing at −24 °C, packing in plastic bags of 0.5 kg and storage at −18° C. By taking control of raw fruit cherries packed in plastic bags.

Fresh fruits and after six months of dry freezing determine soluble substances – refractometer (Naichenko, 2001), tanning and coloring substances by Neubauer and Leventhal methods (Naichenko, 2001), ascorbic acid by iodometric method (Naichenko, 2001), antioxidant activity method FRAP (Hasanov, 2004), water-retaining capacity by weighing on scales. The weight of the fruit for the analysis of 2kg., threefold repetition. Mathematical data processing was performed (Dospehov B.A., 1979) with the program “Excel 2000” and “Statistica”.

3.Results and discussions

As seen from Table 1, content of dry soluble substances in fresh fruits cherry variety Shpanka is 16.1%, which is 1.1% higher than in the fruit variety Lotovka, due to the peculiarities of the variety. After six months storage of frozen cherries fruit, the content of dry soluble substances in processed fruit cherries increased by 2–28%, which is due with holding them in solution of sugar or adding ascorutin or chitosan and passing diffusion process.

Table 1. The content of some components of the chemical composition and water-retaining capacity cherry fruit varieties Shpanka and Lotovka

Type of treatment		Dry soluble substances,%	Tanning and coloring, substances,%	Ascorbic acid, mg /100 g	Antioxidant activity, mmol/dm^3	Water-retaining ability,%
fruit variety of Shpanka						
To freeze		16.1±0.2	0.67±0.02	17.6±0.2	27±2	-
After freezing:	No treatment (control)	16.1±0.3	0.51±0.03	11.0±0.2	23±3	12.2±1.1
	20% sugar solution with the addition of 4% ascorutin	16.4±0.3	0.60±0.02	11.0±0.3	24±2	11.4±1.2
	20% sugar solution with the addition of 1% chitosan	20.0±0.2	0.65±0.01	17.6±0.2	27±1	8.4±1.2
fruit variety of Lotovka						
To freeze		15.0±0.2	1.04±0.02	20.0±0.2	18±2	-
After freezing:	No treatment (control)	15.0±0.1	0.88±0.01	13.3±0.2	14±1	13.2±1.2
	20% sugar solution with the addition of 4% ascorutin	15.4±0.2	0.96±0.02	13.4±0.3	15±2	12.6±1.1
	20% sugar solution with the addition of 1% chitosan	9.2±0.1	1.03±0.03	20.0±0.1	18±1	9.3±1.3
LSD_{05}		0.4	0.4	0.7	0.7	0.4

The content of tannins and coloring substances in frozen fruit cherries decreased slightly. Specifically, in control version it decreased by 23% for fruit cherry varieties Shpanka and to 15% for fruit cherry varieties Lotovka, compared with fresh fruit. While at the processed fruit it decreased by 3 and 10%. Obviously, for the preservation of content tanning and coloring substances contributed of pretreatment before freezing. Confirming the results of research of Scibisz and Mitec (2007) and show the loss of phenolic compounds after six months of freezing is amounted to 20–50%.

Compared to fresh fruits, ascorbic acid content decreased by 37.5%, except for fruit cherries, processed 20% solution of sugar with the addition of 1% chitosan, where the content of ascorbic acid remained at control variant. The results of research of Ancos et al. (2000) is indicate, that after freezing ascorbic acid content is reduced by 55% .

Antioxidant activity of cherry fruit is caused by presence of tannins and coloring matter and ascorbic acid in fruit. As seen from the table, it depends from varieties and for cherries fruit varieties of Shpanka is 27mg/dm^3 and Lotovka is 18 mmol/dm^3. After six months of freezing it decreased by 14 and 22%. While the processed fruit cherry varieties Shpanka and Lotovka in 20% sugar solution with the addition of 4% ascorutin to 11 and 17%. With that, loss was lowest for fruit cherries, processed by 20%

solution of sugar with the addition of 1% chitosan. That also confirms the results research of Scibisz and Mitec (2007), that freezing does not result in significant losses of antioxidant activity.

Water-retaining capacity of cherry fruit after pretreatment by solution and six month of freezing is ranges from 8.4% to 13.2%.

And depending on the variety, it was different – for fruit variety of Shpanka it was 12.2% and Lotovka – 13.2%.

With that, the least she remained for fruit cherries, pretreated with 1% chitosan solution and frozen in plastic bags.

Processing of the data using statistical methods will show a pattern of numerical characteristics. Using of the correlation coefficient we can establish the degree of influence on the quality characteristics of water-retaining capacity and antioxidant activity. Between antioxidant properties and tannins and coloring substances (Table 2) a relationship are strong (r = –0.77). Also, between the water-retaining capacity and ascorbic acid – relationship is strong r = – 0.71.

Therefore, according to a matrix of pairwise correlations on water-retaining capacity has significant influence content of ascorbic acid and on antioxidant activity – the content of tannins and coloring substances.

Results of conducted analysis reflected in the graph (figure 1).

The conducted research has established the advantages of pre-processing. If the antioxidant activity is showing as a dependent variable at (Table. 3) so, we get a linear dependence – $y = 28.7-29.48X_1+0.99X_2$ (where X_1 – the content of tannins and color substances, % X_2 – content of ascorbic acid, (mg / 100 g). As a result of research has been established, that the antioxidant activity of frozen fruit cherry varieties Shpanka and Lotovka previously treated with a sugar solution with the addition of chitosan and ascorutin, particularly in the solution treated by chitosan – have a higher content of tanning and coloring substances – 0.65% and 1.03% and ascorbic acid 17.6% and 20 mg/100g. Conducted studies have established the benefits of treatment before freezing of fruits cherry in 20% sugar solution with the addition of 1% chitosan.

Table 2. The matrix of pairwise correlations between certain indicators of chemical composition and ability moisture-containing of frozen fruit cherries

Indicator	**Dry soluble substances**	**Tanning and coloring substances**	**Ascorbic acid**	**Antioxidant activity**	**Water-retaining capacity**
Dry soluble substances	1	− 0.13	0.39	0.45	0.07
Tanning and coloring substances	− 0.13	1	0.59	**−0.77**	−017
Ascorbic acid	0.39	0.59	1	0.03	**−0.71**
Antioxidant activity	0.45	**−0.77**	0.03	1	−0.36
Water-retaining capacity	0.07	−0.17	**−0.71**	−0.36	1

A) B)

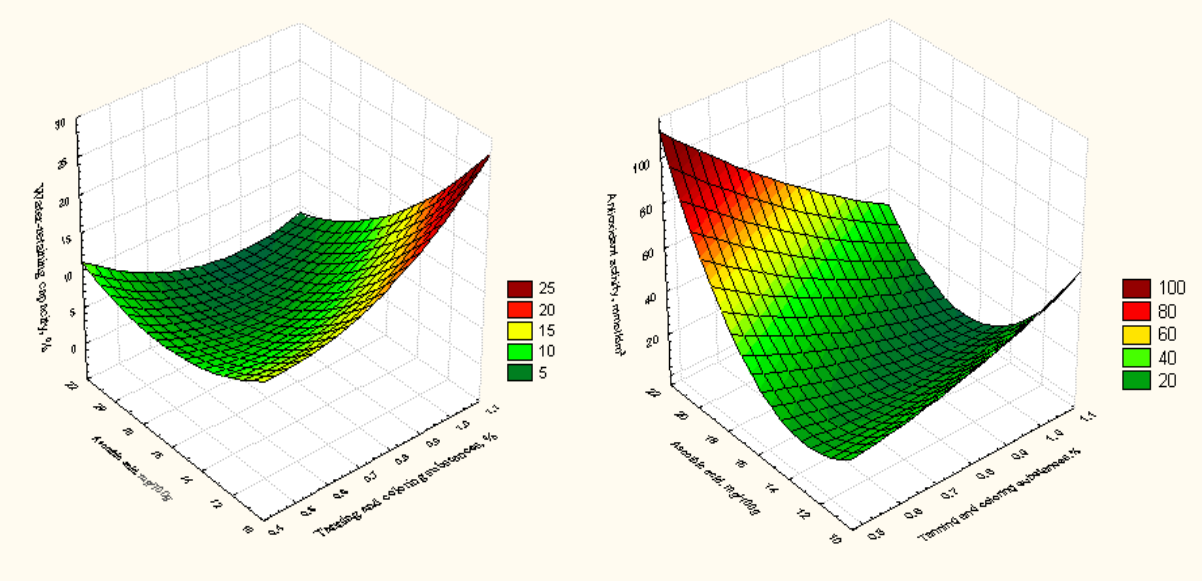

Figure 1. Dependence of water-retaining capacity (A), antioxidant activity (B) from content tanning and coloring substances and ascorbic acid of fruit cherries

Table 3. Results of regression analysis

N=8	Beta*	Std.Err. of Beta*	B*	Std.Err. of B*	t(5)*	p-level*
Intercept			28.7031	2.742152	10.46735	0.000137
Var 2	−1.19020	0.143473	−29.4835	3.554100	−8.29563	0.000416
Var 3	0.72337	0.143473	0.9952	0.197380	5.04188	0.003961

Note* β − coefficient equation shows,in how many units of standard deviation will change the dependent variable by changing one standard deviation of the independent variable;

B − coefficient regression;

Std.Err of Beta − standard error of regression coefficients;

T t-criterion for regression coefficients;

p-level −the probability of the null hypothesis for the regression coefficients.

4.Conclusions

Thus, pretreatment fruit cherry 20% sugar solution with the addition of 1% solution of chitosan are among the most effective test solution and contributes at the level of fresh fruits cherries, content of tanning and coloring substances and ascorbic acid. The antioxidant activity of this fruits is 18–27 mg/dm^3.

Based on correlation and regression analysis obtained mathematical model of determining antioxidant activity of fruit cherries.

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FUNCTIONAL AND PREBIOTIC PROPERTIES OF ACETYLATED ARENGA STARCHES WITH DIFFERENT DEGREE OF SUBSTITUTION

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ABSTRACT

Arenga starch obtained from the pith of sugar palm (*Arenga pinnata*) and subjected to chemical modification by acetylation were produced acetylated arenga starches (AAS) with different degree of substitution (DS). The objective of this research was to evaluate the functional properties and in vitro fermentation of potential prebiotic of native arenga starch (NAS) and AAS with DS 0.039, DS 0.078 and DS 0.139. The products were functional properties including resistant starch (RS) content, binding of bile acids, reduction of cholesterol, and potential prebiotic including the bacterial growth, prebiotic index (PI), changes of pH and short chain fatty acids (SCFA). Functional properties such as the RS and bile acids binding were increased with increasing in DS and the total cholesterol decreased with the increasing in DS. Evaluation of prebiotic property in fecal batch culture was performed and it was found that the AAS produced prebiotic property resulted in a significant high increase in bifidobacteria and lactobacilli, whereas that the bacteroides and clostridia populations were low growth rate. The substrates that showed the positive PI were all sampled of NAS and AAS. The pH drops rapidly during the first 12 h and then further was found to constant in all of the NAS and AAS media. All the AAS produced a significant increase in SCFA production indicating they were fermented by the gut microbiota. In conclusion, the AAS were can be used as an alternative or complementary treatment in hypercholesterolemia and fermented by gut bacteria and exhibited potential to be used a novel source of prebiotics.

1. Introduction

The starch in its native form has limited of non-food applications, therefore it does not always have the desired properties for certain types of processing but when it modifications, it can increase its range of use. The chemically modified of the starch by acetylation has been widely investigated (Bello et al., 2010; Luo & Shi, 2012; Mbougueng et al., 2012; Singh & Nath, 2012). The acetylation may be performed to improve the physical, chemical and functional properties of the starch (Xu et al., 2004). In the acetylation process, the hydroxyl groups of the glucose monomers are converted to acyl the groups CH_3COO- (Graaf et al., 1995), therefore the acetylation is an esterification of hydroxyl groups in the anhydroglucose unit of the starch molecule

(Huang et al., 2007). Acylated starch with low degree of substitution (DS) is commonly obtained by esterification of native starch in an aqueous medium in the presence of an alkaline catalyst.

The applicability of acetylated starches was completed dependent on the DS, the starch acetates, in accordance with DS, are classified as low DS (<0.1), medium DS (0.1-1.0) and high DS (>1.0) acetated. There is a commercial interest in starches with a DS of 0.01-0.20 because of their use based on properties to film formation, adhesion, thickening, stabilizing and texturizing (Boutboul et al., 2002; Matti et al., 2004; Shingh et al., 2004; Sodhi &Shingh, 2005). Starch acetates are prepared commercially with a low (< 0.1) DS through the reaction of an aqueous suspension of starch granules with acetic anhydride (Biswas et al., 2008).

Acylated starches with low DS content of resistant starch (RS) plays important physiological roles and has the potential to improve human health and lower the risk of many diet-related diseases. RS is explained as that fraction of starch, which escapes digestion in the small intestine and passes into the large intestine where it is more or less fermented by gut microflora. It is considered to be a functional component of food due to the health advantages it confers following its consumption. Many investigates suggest that RS intake decreases postprandial glycaemic and insulinemic responses (Li et al., 2010), lowers plasma cholesterol and triglyceride concentrations (Liu & Ogawa, 2009), increases satiety and decreases fat storage (Chiu & Stewart, 2013). RS as a prebiotic can promote the growth of beneficial microorganisms such as bifidobacteria and lactobacilli, what exert a lot of beneficial effects on human body.

RS of acetylated starches fermentation produces in the large intestine are generally recognized as beneficial for maintaining host homeostasis. The short chain fatty acid (SCFA) are fermentation end-products of the intestinal microbiota that make use of exert an extensive influence on host physiology through nutritional, regulatory, and immune-modulatory properties. Moreover, SCFA act as signals for the regulation of virulence genes in enteric pathogens. It had been reported that as a whole, SCFA acidify the luminal pH, which compresses the growth of pathogens, SCFA also influence intestinal mortality (Sun & Riordan, 2013; O'connor, 2013). Dass et al. (2007) has been reported that among SCFA, acetate is especially seen as a lipogenic compound while propionate acts as a glucogenic substrate and an inhibitor of lipogenesis. The butyrate provides as a major energy substrate as well as a regulator of cell growth and differentiation (Hong et al., 2005; Ge et al., 2005), butyrate may reduce the risk of colon cancer by stimulating apoptosis of colonocytes (Furusawa et al., 2013; Gao et al., 2009). In the current study, acetylated starches were prepared by reacting arenga starch with acetic anhydride in aqueous slurry to evaluated the effect of DS on the functional properties and prebiotic potential of acetylated arenga starches.

2. Material and methods

2.1.Materials

Arenga starch (*Arenga pinnata* Merr.) used for this study was obtained from Sigi distric Central Sulawesi Province, Indonesia. High-purity acetic anhydride 98%, sodium hydroxide (NaOH) and hydrochloric acid (HCl) were purchased from Merck. The chemicals for analysis used in the study were of analytical grade purchased at local agent.

2.2. Preparation of acetylated arenga starch

The acetylated arenga starches (AAS) was prepared by a modified procedure of Phillips et al. (1999) with slight modification. The DS was determined by a titration method. Starch (100 g) was dispersed in 225 mL of distilled water and stirred for 60 min at 25^{o}C.

The suspension was adjusted and controlled at pH 8.0 ± 8.2 with 3.0% NaOH solution. Acetic anhydride of (5, 10, 15 % starch base, sb) was slowly added drop-wise to the stirred slurry. After the complete addition of the acetic anhydride, the reaction was continued for 60 min and interrupted, setting the pH at 4.5 with 0.5 N HCl. The suspension was precipitated for 10 min and subjected to two successive washes twice with distilled water and once with 95% ethanol, and then oven-dried at 40^{o}C for 48 h or up to approximately 12% moisture, and ground on an analytical mill. Acetylation of starch with 5% (sb) acetic anhydride gave the AAS with DS 0.039, with 10% (sb) acetic anhydride gave that with DS 0.078, and with 15% (sb) acetic anhydride gave that with DS 0.139.

2.3. Determination of resistant starch

The RS determined as a dietary fiber according to the enzymatic gravimetric method (Ebihara et al., 2006). To determine the content of RS, 4 g of sample was suspended in 160 mL of 0.08 M phosphate buffer (pH 5.5) before the addition of 40 µl of heat-stableα-amylase. The solution was incubated at 95°C for 75 min and then allowed to cool. The pH of the solution was adjusted to 4.5 by adding 1 N HCl, and the sample was incubated with amyloglucosidase at 60°C for 45 min. Aliquot (100 µl) was added with 10 mL of glucose oxidase reagent and the mixture was incubated at 20°C for 20 min. Absorbance was measured using a spectrophotometer at 510 nm. The concentration of RS was calculated as follow: RS (g/100 g) = (1 – G × 0.9 / wt. sample) × 100, where wt. sample was the initial weight (g), and G is the weight of glucose (g).

2.4. The bile acids/salts binding

The binding of bile acid/salts (cholic acid, sodium taurocholic sodium deoxycholic) was measured by *in vitro* analysis (Smietana et al., 2000). The sample (100 mg) was admixed with 10 mL of solution of each bile acid. The solutions were prepared in 0.1 mol phosphate buffer pH 7.6 for each bile acid in 2 µmol/mL concentration. The samples and parallel blank samples were incubated at 37°C for 30 min. Centrifugation was performed at 2000 g for 5 min. The sample (50 µl) was combined with 5 mL 70% sulphuric acid and 1 mL of freshly prepared furfural solution (2.3 g/L) with careful mixing of the whole sample. Absorbance was measured at 510 nm after 80 min. The results were expressed as percent of bile acid absorption.

2.5. The cholesterol binding

The cholesterol binding was measured by *in vitro* analysis (Smietana et al., 2000). The sample (100 mg) was combined with 2 mL emulsion composed of 1% lecithin, 1.375% sodium salt of deoxycholic acid and 0.225% cholesterol prepared in 0.1 mol phosphate buffer of pH 6.8. Incubation for 1 h at 37°C was performed in a shaking water bath. Cholesterol absorption by 20 µl emulsion was analysed using reagent kits. The results were expressed as percent of cholesterol adsorbed by samples.

2.6. Preparation and operation of batch fermenters

Fresh faecal samples from a healthy human volunteer who had no history of antibiotics treatment in the previous 3 months were first diluted (1:10) in anaerobic buffer containing 0.1 M phosphate-buffered saline (PBS) solution, pH 7.0, and then were homogenized using a magnetic stirrer at normal speed for 2 min. A 10 mL portion of the filtered faecal slurry was added to 90 mL growth medium in 250 mL batch fermentation vessels. The growth medium contained the following ingredients: peptone water (2 g/L), yeast extract (2 g/L), NaCl (0.1 g/L), K_2HPO_4 (0.04 g/L), KH_2PO_4 (0.04 g/L), $MgSO_4.7H_2O$ (0.01 g/L), $CaCl_2.6H_2O$ (0.01 g/L), $NaHCO_3$ (2 g/L), Tween 80 (2 mL/L), Hemin (0.05

g/L), Vitamin K (10 µL/L), L-cysteine (0.5 g/L) and bile salts (0.5 g/L). The NAS and BAS (1% w/v) were added to each vessel just before addition of fecal slurry (10% w/v). The vessels were maintained under anaerobic conditions by continuous sparging with oxygen-free nitrogen. Temperature was automatically controlled at 37 °C. The batch experiments were performed in duplicate with two different fecal donors for each substrate. At each experiment a 5 mL sample was taken from each vessel at 0, 12, 24, and 48 h for analysis (Vardakou et al., 2008; Ramnani et al., 2012).

2.7. Bacterial enumeration

The samples from each vessel were immediately transferred to an anaerobic cabinet containing an atmosphere of 10% H_2, 10% CO_2, and 80% N_2, and were serially diluted with pre-reduced half strength peptone water, pH 7, supplemented with 0.5 g cysteine–HCl L^{-1}. Portions of 1 mL from each dilution were plated, in duplicate, onto agar plates (incubation anaerobic condition at 37ºC for 48 h). The selective growth media used were total plate count, for total bacterials; trypticase soy agar, supplemented per litre with 75 mg kanamycin, 5 mg haemin, 75 mg vancomycin and 50 mLlaked horse blood, for *Bacteroides* spp.; reinforced clostridial agar, supplemented per litre with 8 mg novobiocin and 8 mg colistin, for *Clostridium* spp.; rogosa agar, supplemented with 1.32 mL glacial acetic acid L^{-1}, for *Lactobacillus* spp.; Columbia agar containing per litre 5 g glucose, 0.5 g cysteine HCl, and 0.5 mL propionic acid, pH 5.0, for *Bifidobacterium* spp. All agars were purchased from Oxoid (Basingstoke, Hants, UK) and prepared according to the supplier's instructions. All the antibiotics used in the preparation of media were purchased from Sigma.

2.8. Calculation of prebiotic index

The equation used to estimate the prebiotic index (PI) values (Palframan et al., 2003) was: PI = (Bif /Total) – (Bac / Total) + (Lac / Total) – (Clos /Total) where Bif, Bac, Lac, Clos, and Total are the numbers of bifidobacteria, bacteroides, lactobacilli, clostridia, and total numbers of bacteria, respectively, at the time of sampling relative to their respective numbers at the time of inoculation. The equation embodies the assumption that an increase in the populations of bifidobacteria and/or lactobacilli is a positive effect while an increase in bacteroides and/or clostridia is a negative effect.

2.9. Determination of pH

The pH of the samples was determined using a digital pH meter (Eutech instruments, Cyber scan) calibrated with buffers at pH 4.0 and 7.0 (Fisher Scientific, UK).

2.10. Determination of organic acid

Culture medium (1 mL) was centrifuged at 4500 x g for 30 min. The supernatant was filtered through a 0.22 mm filter into a 1.5 mL eppendorf tube for storage at 4ºC until use. Samples of 1 µL were injected into a high-resolution gas chromatography (GC Shimadzu 8A) equipped with a flame ionization detector and an HP Innowax 19091 - 136 column (GP 10%-SP 1200/1% H_3PO_4 on 80/100 mesh chromosorb WAW, 60m x 0.250 mm). The carrier gas was nitrogen with a flow rate of 1.8 mL/min, and the split ratio was 40:1. The oven temperature was maintained at 90ºC for 0.5 min, and then increased to 110ºC at a rate of 10ºC/min, increased to 170ºC at a rate of 5ºC/min and finally increased to 210ºC at a rate of 20ºC. Injector and detector temperatures were 230ºC. SCFA mixture containing acetate, propionate and butyrate at specific concentration were used as standard.

2.11. Statistical analysis

The data experiments were analyzed using SPSS (version 17.0) software and subjected to one-way analysis variance. The difference of means between groups was also analyzed using Duncan's multiple comparison test. The level of $P < 0.05$ was considered as significant. All experiments were repeated once or twice at later dates.

3.Results and discussions

3.1. Resistant starch

The RS content of both native arenga starch (NAS) and the AAS with different DS tended to increase with increasing DS (Figure 1). The result shows that the enhanced acetylation of starch considerable increased the level of RS. This is because the introduction of acetyl groups and long carbon chains to the starch molecules increased stereospecific blockade, which has a covering effect for starch molecules, thus being able to delay the enzymatic degradation. Previous studies reported that RS content of butyrylated arenga starches (Rahim et al., 2012) and octenyl succinylated corn starches (Wang et al., 2011), increased with increasing the DS. All substituted arenga starches had a higher resistance to enzyme hydrolysis, compared to the native starch. The results suggested that AAS had more influence on resistance to enzyme than the native starch.

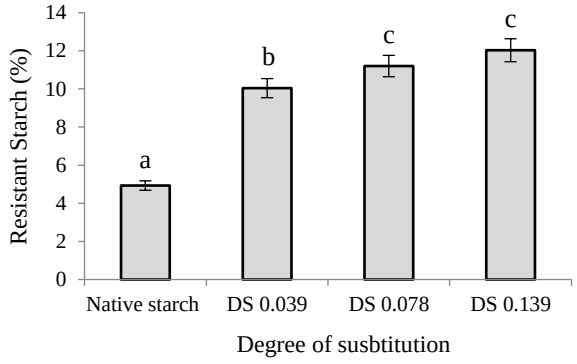


Figure 1. Effects of DS on the RS content of NAS and AAS. All values are expressed as mean±SD. Means with different letters in a row are significantly different at $\alpha=0.05$ by Duncan's multiple range test.

3.2. Binding of bile acid/salts

The data on the bile acid/salts absorption are presented in Figure 2. This data indicated the affinity to cholic acid, sodium taurocholate and sodium deoxycholate of AAS trended to increase with increasing DS. The bile acid/salts binding capacity of AAS significantly increased compared to that of NAS. These were similar to the results of Souza et al. (2015) have been reported that bile acid binding capacity were increased by acetylation of β-glucan. The interaction of AAS with bile acid/salts were possibly chemical binding by hydrophobic interactions and entrapment in a gel matrix resulting by acetylation. Thus, it makes sense that removing bile acid/salts directly from circulation or reducing the reabsorption of bile acid/salts, either by forming a complex with of AAS or entrapping bile acid/salts in the viscous matrix, would have health beneficial. Thongngam & Clements (2005) which support the binding of bile salt (sodium taurocholate) with chitosan by hydrophobic interactions and Cornfine et al. (2010) has been reported that the binding of bile acids with dietary fiber of lupin acetate by hydrophobic interactions.

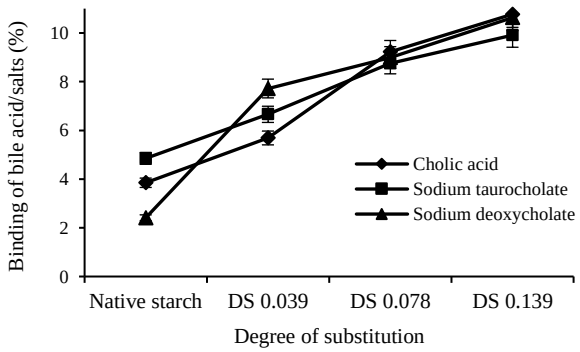


Figure 2. Effects of DS on the binding of bile acid/ salts of NAS and AAS. Vertical bars represent the standard deviation of the means.

3.3. Reduction of total cholesterol

The total cholesterol data are presented in Figure 3. This data showed that the total cholesterol of AAS decreased with increase in DS, which is in accordance to the ability to bind the bile acid/salts and increasing viscosity. This is due to incorporation of acetyl groups by acetylation which assisted to the increase in cholesterol binding. Similar to acetylated ß-glucan have been shown to lower blood cholesterol, though these prebiotics including the resistant starch (Vulevic et al., 2013). In parallel, a number of human feeding studies with well-powered cohorts of hypercholesteroleamic individuals have demonstrated that probiotics selected for bile salt hydrolase activity lower plasma cholesterol levels to asimilar extent to oats and acetylated ß-glucan (Jones et al., 2012). Soluble dietary fibres were (1.3:1.4) beta-D-glucan have been reported to lower plasma cholesterol contents in the human body, at least in part by averting bile acid/salts from being reabsorbed into the enterohepatic circulation (Gunness et al., 2010).

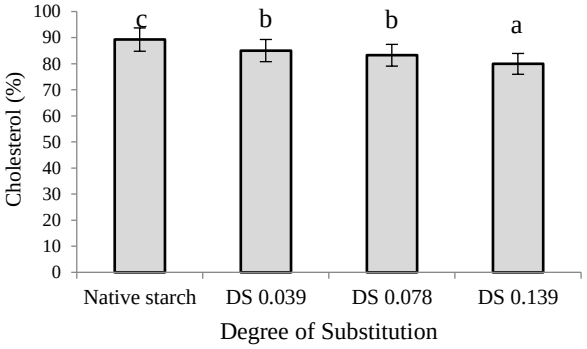


Figure 3. Effects of DS on the cholesterol content of NAS and AAS. All values are expressed as mean±SD. Means with different letters in a row are significantly different at α=0.05 by Duncan's multiple range test.

Table 1. Bacterial population (log CFU/mL) in batch cultures at 0, 24, and 48 h in presence of NAS and AAS with DS 0.039, DS 0.078 and DS 0.139

Incubation time (h)	Substrates	Bacterial population (log CFU/mL)*				
		TB	Bif	Bac	Lac	Clos
0	NAS	6.30 ±0.17	6.02 ±0.04	5.00 ±0.02	4.82 ±0.08	4.13 ±0.15
	DS 0.039	5.99 ±0.14	6.07 ±0.03	5.06 ±0.04	4.70 ±0.06	4.13 ±0.19
	DS 0.078	5.97 ±0.07	5.91 ±0.04	5.67 ±0.04	4.81 ±0.07	4.51 ±0.00
	DS 0.139	6.03 ±0.06	5.75 ±0.13	5.03 ±0.03	4.83 ±0.03	4.10 ±0.22
24	NAS	9.00 ±0.01	8.24 ±0.07	6.05 ±0.01	7.86 ±0.04	5.90 ±0.06
	DS 0.039	9.08 ±0.00	8.14 ±0.16	5.91 ±0.06	7.96 ±0.05	6.06 ±0.07
	DS 0.078	8.99 ±0.06	8.08 ±0.05	5.83 ±0.04	7.90 ±0.06	6.07 ±0.02
	DS 0.139	8.92 ±0.02	8.06 ±0.07	5.76 ±0.04	7.86 ±0.00	6.00 ±0.01
48	NAS	8.49 ±0.08	7.71 ±0.06	5.68 ±0.04	7.74 ±0.02	6.01 ±0.01
	DS 0.039	8.49 ±0.06	7.84 ±0.03	5.93 ±0.03	7.77 ±0.04	6.22 ±0.16
	DS 0.078	8.57 ±0.06	7.83 ±0.02	5.69 ±0.08	7.77 ±0.04	6.34 ±0.00
	DS 0.139	8.57 ±0.05	7.77 ±0.01	5.69 ±0.03	7.74 ±0.00	6.18 ±0.18

* Data reported are average values ± standard deviations. NAS, native arenga starch; AAS, acetylated arenga starches; TB, total bacteria; Bif, Bifidobacterium; Bac, Bacteroides; Clos, Clostridium; and Lac, Lactobacillus.

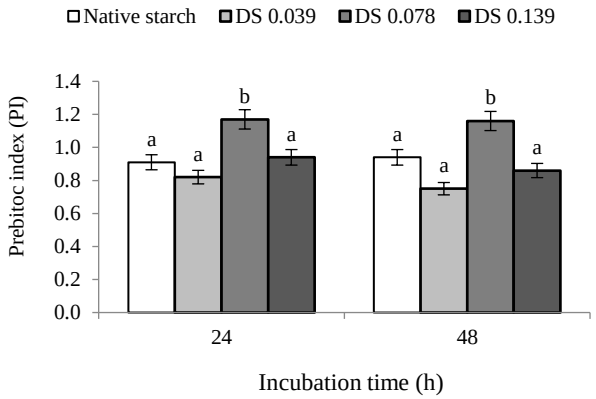
3.4. Modulation of bacterial population

The effect of the addition of NAS and AAS with DS 0.039; DS 0.078 and DS 0.139 on the bacterial populations after 0, 24 and 48 h of incubation in batch culture fermentors are shown in Table 1. These results indicate that the supplement of AAS to the batch fermenter resulted in a significant increase in the levels of bifidobacteria and lactobacilli, whereas the levels of bacteroides and clostridia were decreased, which are considered to have beneficial properties. The bifidobacterial and lactobacilli numbers increased by two or three log sickle from incubation time 0 - 24 h, longer incubation time from 24 – 48 h resulted constant, whereas bacteroides numbers constant and lactobacilli numbers increased by three log sickle from incubation time 0 – 24 h, longer incubation time from 24 - 48 h were constant for all of the samples.

The results indicated that NAS and AAS had biggest effect on the bacterial population of bifidobacteria and lactobacilli, and its had slightly or no effect on the other bacterial populations of bacteroides and clostridia. Lesmes et al. (2008) has shown that even thermally produced polymorphs of the same type of RS can have differing effects on the gut microbiota, with one increasing atopobium whilst the other increased bifidobacteria. Previous studies have indicated the enhancement of bifidobacteria and lactobacilli, the main targets of prebiotic action in presence of the RS (Salazar et al., 2009; Ramnani et al., 2010).

3.5. Prebiotic index

The prebiotic property of the NAS and AAS produced was evaluated by in vitro fecal batch culture. The PI represents a comparative relationship between the growth of beneficial bacteria, such as bifidobacteria and lactobacilli, and that of the undesirable ones, such as bacteriodes and clostridia. In this study, the majority of the bifidobacteria and lactobacilli grew better on the AAS compared to the other selected gut bacteria. On the other hand, all of the bacteriodes and clostridia species shown very low growth on the AAS susbtrates. This cleary indicates that the AAS may allow a prebiotic effect by preferentially increasing the growth of bifidobacteria and lactobacilli, and decreasing the growth of bacteriodes and clostridia. The substrates that showed the positive PI were all substrates (native, DS 0.039, DS 0.078 and DS 0.139) suggest that it could be the better prebiotic (Figure 4). The positive PI value is therefore, determined by the proportion of desired bacterial groups (in this case bifidobacteria and lactobacilli) versus less desirable bacterial groups. Variation of PI value may cause by levels of acetylation, DS and molecular weight distribution. It had been reported that PI of GOS has positive value however its values were varied in range of 3.5-7.2 for degree of polymerization (DP) 3 and 4, respectively (Li et al., 2015). Ladirat et al. (2014) indicated that GOS, especially its large size-fractions (DP 4-7), espouse the recovery of bifidobacteria whereas low size-fractions (DP 2-3) preferred metabolized by non-amoxicillin treated human fecal inoculums.



Figures 4. PI scores from batch cultures 24 h and 48 h using NAS and AAS. All values are expressed as mean±SD. Means with different letters in a row are significantly different at α=0.05 for each time by Duncan's multiple range test.

3.6. The pH changes along fermentation

The decrease of the pH along fermentations between 0, 12, 24 and 48 h are shown in Figure 5. In all cases, pH drops rapidly during the first 12 h and then further decreases slowly at longer fermentation was found to constant in all of the NAS and AAS media. In agreement with related studies (Kabel et al., 2002; Moura et al., 2008), two periods can be distinguished: in the first stage (lasting about 14 h), the pH decreased meaningfully, whereas pH remained fairly constant through fermentation times. Since all the experiments were performed under uncontrolled pH conditions, the rapid drop in pH in media fermentations was due to the accumulation of SCFA and other organic acids.

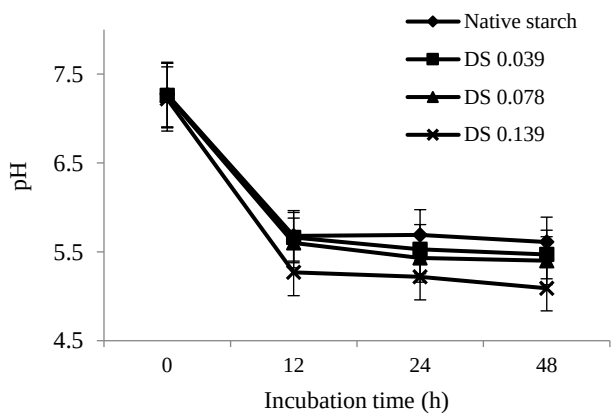


Figure 5. pH in batch cultures at 0, 12, 24, and 48 h in presence NAS and AAS. Vertical bars represent the standard deviation of the means.

3.7. Production of short chain fatty acids

The concentration of total, acetate, propionate, butyrate acid produced in batch cultures at 0, 24, and 48 h of NAS and AAS with DS 0.039, DS 0.078 and DS 0.139 are shown in Table 2. All the substrate from AAS produced a significant increase in total, acetate, propionate and butyrate production suggesting they were fermented by the gut microbiota. During the period 0 - 24 h, the SCFA concentration increased simultaneously to the results in a marked pH drop to around 5.5 for all samples expected DS 0.139. The SCFA production were dominated by acetate, followed propionate and butyrate. The structural changes of the AAS during degradation may influence fermentation patterns, including SCFA composition. In the early stages of fermentation, most of the AAS are present as different oligosaccharide, linkage composition and DP, which are not readily available as energy source. Therefore, the microbiota tended to produce acetate because the conversion of sugars into acetate yields more energy than conversion into other SCFA. In previous studies Alonso et al. (2011) reported that glucooligosaccharides, galactooligosaccharides, xylooligosaccharides and arabinooligosaccharides were completely depleted after 21 h. At this fermentation time, the major organic acids were acetate and butyrate (molar ratio, 1:0.22), the minor amounts of formiate were also present in the medium. On the other hand, fermentation times longer than 14 h resulted in depletion of lactate and succinate: both acids are intermediary products of carbohydrate fermentation, and can be converted into acetate, propionate and butyrate by common intestinal bacteria (Belenguer et al., 2006; Gullón et al., 2010).

Tabel 2. SCFA concentration (mM) in batch cultures at 0, 24, and 48 h in presence NAS and AAS with DS 0.039, DS 0.078 and DS 0.139.

Incubation time (h)	Substrates	SCFA (mM)				Molar Ratio AA:PA:BA (%)
		Acetic acid (AA)	Propionate acid (PA)	Butyrate acid (BA)	Total SCFA	
0	**NAS**	0.257	0.955	0.471	1.683	15:57:28
	DS 0.039	1.041	0.978	0.492	2.511	41:39:20
	DS 0.078	1.032	0.990	0.513	2.535	41:39:20
	DS 0.139	1.073	0.976	0.451	2.500	43:39:18
24	**NAS**	6.986	2.562	1.022	10.570	66:24:10
	DS 0.039	7.800	1.629	0.701	10.130	77:16:7
	DS 0.078	8.046	2.306	0.913	11.265	71:21:8
	DS 0.139	8.481	2.457	0.982	11.920	71:21:8
48	**NAS**	1.873	0.562	Nd	2.435	77:23:0
	DS 0.039	1.068	0.210	Nd	1.278	84:16:0
	DS 0.078	3.074	1.325	0.859	5.256	59:25:16
	DS 0.139	1.034	1.213	0.574	2.821	37:43:20

Nd: Not detectable

4. Conclusions

The acetylation of arenga starch promoted the incorporation of acetyl groups in the molecule, improving the functional and prebiotic properties of the modified starches and resulting AAS in a DS allowing food application. The RS content and the bile acid/salts binding of AAS tended to increase and reduce the cholesterol with the increase of DS, which were equally important functional properties.On the basis of the data obtained through in vitro fermentations NAS and AAS exhibited a potential to be used as an effective source of prebiotic, increasing the populations of bifidobacteria and lactobacilli, and decreasing the numbers of bacteriodes and clostridia. The all of the AAS were fermentable by gut microbiota as indicated by noticeable increases in SCFA and reduction in the pH of media. It could be suggested that the prebiotic may be useful and can be used as an alternative or complementary treatment in hyperlipidemia and related disease conditions.

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SALGAM POWDER PRODUCTION FROM FERMENTED SALGAM: A TRADITIONAL TURKISH LACTIC ACID BEVERAGE

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ABSTRACT
The aim of this study was to produce instant salgam powder from fermented salgam and to investigate some physicochemical and sensorial characteristics of the final product. Firstly, fermented salgam was produced by traditional method and then moisture of produced salgam was removed by freeze-drying for the production of powder form of salgam. Then freeze-dried salgam was reconstituted by water at different ratios (1.5, 2.5, 3, 3.2 and 3.5 % powder) for the production of instant salgam from powder. According to the pH, total titratable acidity and salt contents, at least 3.2 % powder was chosen as the most suitable reconstitution ratio that fulfills the requirements in the TS 11149 salgam standard. Then for comparing reconstituted salgam (3.2 %) with fermented salgam, pH, total titratable acidity, salt, total solid matter, total phenol content, anthocyanin content, turbidity, colour characteristics (L*, a*, b*) and sensorial analyses were performed on the fermented and reconstituted salgam samples. According to the results of the sensorial analyses, instant salgam had close score to the fermented salgam from the sensorial point of view and preferred by panelists as the ready-to-use product.

1.Introduction

Fermentation is one of the oldest methods used by humankind for the production and preservation of foods. Besides production and preservation, fermentation has many positive effects on foods. It increases nutritional value of the foods, enhances functionality and sensory properties of the final products (Hutkins, 2006; Erten *et al.*, 2016). Fermented foods are produced on a large scale in industry besides the production on a small scale at homes traditionally. Pickles, table olives and sauerkraut are important industrial fermented food products produced mostly on a large scale. Also there are some local fermented foods that are specific to certain geographic regions (Erten *et al.*, 2017).

Salgam is a local lactic acid fermented beverage. It is defined by Turkish Standards Institution (TSE) in TS 11149 standard as "the product produced by lactic acid fermentation of turnip (*Brassica rapa*), black carrot (*Daucus carota*), sometimes chili powder and extract that is obtained from the first lactic acid fermentation of bulgur flour, sourdough, drinking water and salt, which can be heat treated for preservation, if desired" (TSE, 2016). Salgam is produced traditionally at homes and also at industrial scale in the southern part of Turkey, particularly in the cities of Adana, Mersin, Kahramanmaras and Hatay. However, it is also very popular and gaining importance in other cities of our country (Erten *et al.*, 2008, 2014; Erten and Tanguler, 2012; 2016). But an important part of salgam is still produced in the southern part and transportation of the

produced salgam to other cities is important for the protection of the end product.

Freezing process is widely used for long-term preservation of vegetables and fruits. Cryopreservation process completely terminates or decreases the biochemical and microbiological activities in vegetables and fruits. Drying technology is the removal of water from foods and providing end-products with a longer shelf life and reduced volume. Therefore, it provides convenience in shipping and handling and contributes to the diversity of products on the market. Freeze-drying technique is a drying technique that contains advantages of both freezing and drying processes. By this technique, it is possible to obtain a high quality end-product with good nutritional quality, texture, flavour and colour. In vacuum freeze-drying technique, foods are firstly frozen at low temperatures and then frozen product is dehydrated by sublimation (Di Matteo *et al.*, 2003; Ratti, 2012). Final product of excellent quality is produced since most of deterioration and microbiological reactions are prevented due to the absence of liquid water and the low temperatures required for the process. Low temperature is also important for the protection of colour, appearance and flavour which are important quality parameters for consumers. The solid state of water during freeze-drying protects the primary structure and the shape of the products with minimal reduction of volume. However, disadvantage of this technique is its high operation cost (Ratti, 2012). With freeze-drying process, juices are converted to powdered form. Volume and weight of them are reduced and that property has some advantages on shipping. By freeze-drying, moisture is decreased and low moisture level enables the storage with a longer shelf life. Also, it is possible to reach freeze-dried foods in four seasons. There are some studies in the literature on freeze-dried juices such as mandarin juice, watermelon juice, pineapple juice, star fruit juice, cornelian cherry juice, strawberry, carrot, pomegranate juice, sour cherry juice, lemon juice, grapefruit juice, orange juice, rosehip and also some other foods such as yoghurt, coffee etc. (Ratti, 2001; Marques *et al.*, 2006; Vadivambal and Jayas, 2007; Koca *et al.*, 2007; Rico *et al.*, 2007; Cam and Ersus, 2008; Erbay et al., 2009; Berk, 2009; Koc *et al.*, 2009; Demiray and Tulek, 2010; Sahin, 2013; Saikia *et al.*, 2014; Oszmianski *et al.*, 2015; Mishra *et al.*, 2015; Tatemoto *et al.*, 2016; Baeghbali *et al.*, 2016; Karaca *et al.*, 2016). However, it is the first study on the production of instant salgam powder by freeze-drying technique.

The aim of this study was the production of instant salgam powder by freeze-drying technique and evaluation of the quality by comparing the physicochemical and sensorial properties of salgam and its powder form.

2. Materials and methods

2.1. Materials

For the production of fermented salgam, black carrot, salgam turnip, bulgur flour (*setik*) and rock salt were used. All raw materials were obtained from local markets. Baker's yeast was used for the production of sourdough by fermenting at room temperature overnight (Vadivambal and Jayas, 2007; Koca *et al.*, 2007; Erten *et al.*, 2008; Mishra *et al.*, 2015). Drinkable water was used for fermentations. Fermented salgam was produced with traditional method according to Erten and Tangüler (2012).

2.2. Fermented salgam production

Production of fermented salgam with traditional method is shown in Figure 1 (Canbas and Deryaoglu, 1993; Erten and Tanguler, 2012). For the first fermentation, also known as dough fermentation, bulgur flour (3 %), rock salt (0.2 %), sourdough

(0.2 %) and adequate drinkable water were mixed and kneaded for the formation of dough. The dough was fermented in a 30 litres of plastic vat at 25°C for 4 days. The fermented mixture was extracted with adequate water for three times. The obtained extracts were used for second fermentation (Erten *et al.*, 2008; Erten and Tangüler, 2016). The extracts obtained from first fermentation were combined with 3 cm of slices of black carrot (20 %), rock salt (1.5 %) and sliced turnip (2 %) to perform the second fermentation, also known as main or carrot fermentation (Tanguler *et al.*, 2014a, b). Adequate drinkable water was added to fill the vat. Fermentation was conducted for 16 days at 25°C. Trials were conducted in duplicate. Fermentations were followed by monitoring pH and total titratable acidity of the salgam samples. The pH was measured by using a pH meter (Mettler-Toledo pH Ion/S220) according to the A.O.A.C. (1990). Total titratable acidity was determined as lactic acid by titration with 0.1 N NaOH (Ough and Amerine, 1988). At the end of the fermentation, fermented product was roughly filtered and stored at 4°C for further analyses.

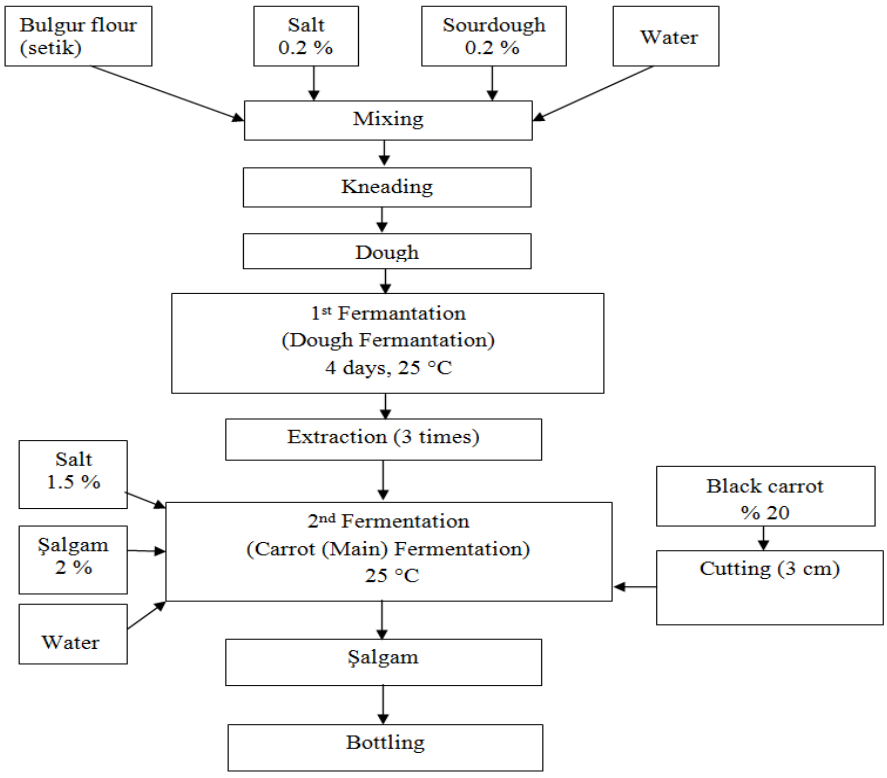

Figure 1. Fermented salgam production by traditional method

2.3. Freeze-drying process

Production of freeze-dried salgam powder is shown in Figure 2. Firstly, samples were frozen at -30 °C for 24 hours in metal trays. Then frozen salgam samples were put into the freeze-drier (Ilshin FD8512, Netherlands) at -66 °C for 60-72 hours under 5 mtorr pressure. At the end of this time, freeze-dried powder salgam was obtained and vacuum packaged (DZ-

300/2SA, China) to protect the samples from humidity during storage. Packaged salgam powder samples were stored at 4°C until analyses.

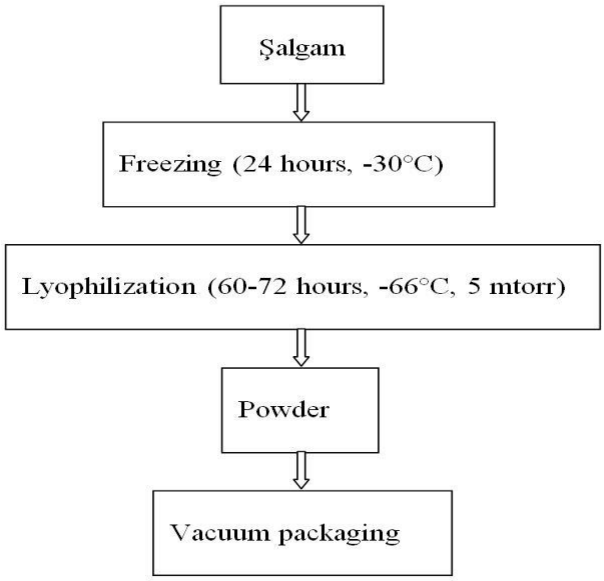

Figure 2. Production of freeze dried salgam powder

2.4. Reconstitution

Freeze-dried salgam was reconstituted by water at different ratios (1.5, 2.5, 3, 3.2 and 3.5 % powder) for the production of instant salgam from powder form. Trials were conducted in duplicate and pH, total titratable acidity and salt analyses were performed on the reconstituted samples. Salt content was analyzed by titration with 0.1 N $AgNO_3$ solution.

2.5. Physicochemical analyses

According to the pH, total titratable acidity and salt contents, the most suitable reconstitution ratio that fulfills the requirements in the TS 11149 salgam standard was chosen. Then some other analyses were performed both on reconstituted and fermented salgam samples to investigate the physicochemical properties of the end product. For this purpose, besides the pH, total titratable acidity and salt analyses, some other physicochemical analyses such as total solid matter, total phenol and anthocyanin contents, colour characterization and turbidity level were done. Also total solid matter of the samples were determined. The total phenolic content of the extract was determined by the Folin–Ciocalteu reagent and determined values were expressed as gallic acid equivalents (Slinkard and Singleton, 1977). For the determination of of anthocyanine compounds, the sodium metabisulfite bleaching method was used (Giusti and Worsltad, 2001). Bleached and unbleached trials of salgam samples were prepeared and then the absorbance difference of bleached and unbleached trials were obtained by Shimadzu UV-1700 model spectrometer at the wavelength of maximum absorbance of anthocyanins. The determined values were expressed as cyanidin 3-glucoside equivalents based on the standart curve of cyanidin 3-glucoside (Ribéreau-Gayon *et al.*, 2000).

Turbidity test was done by turbidimeter (WTW Turb 550 model). Colour of the samples were characterized by measuring chromatic values as L*, a* and b* by Hunter Lab colorimeter (ColorQuest XE Hunter

Lab) was used (Hunter, 1975; Gould, 1977). Chroma (ΔC) and hue angle (Hue) values calculated based on the following equations 1 and 2 as illustrated below:

$$Hue = [arctan\,(b^*/a^*)] \quad (1)$$

$$\Delta C = \sqrt{(a^{*2} + b^{*2})} \quad (2)$$

2.6. Sensory evaluation

Reconstituted salgam samples were compared with fermented salgam samples from the sensorial point of view by panelists. A total of 11 panelists evaluated the end products for colour, odour and flavour, taste, acidity (sourness) and overall sensory impression by using 10-point hedonic scale ranging from 1 (dislike extremely) to 10 (like extremely). Also preference ranking test (1-5) was used (Altuğ and Elmacı, 2005).

2.7. Statistical analysis

Results were subjected to analysis of variance. Duncan's multiple range test was applied for significant differences by using SPSS 20 statistical software.

3. Results and Discussion

Fermented salgam was produced by traditional method. Fermentation was monitored by determining pH and total titratable acidity values and terminated when acidity was stable. Total titratable acidity of the fermented salgam during fermentation is shown in Figure 3. At the beginning of the fermentation, total acidity as lactic acid were 0.089 g/L. At the end of the fermentation, it reached to 9.16 g/L. Total acidity value increased during 14 days and then stayed stable and at the end of the 16th day, fermentation was terminated. The pH of the fermented salgam at the end of the fermentation was 3.62. pH and total acidity results of the present study were in agreement the previous studies, ranging from 3.26 to 3.58 and 6.27 to 8.89 g/L, respectively (Erten *et al.,* 2008; Tanguler 2010; Agirman, 2014).

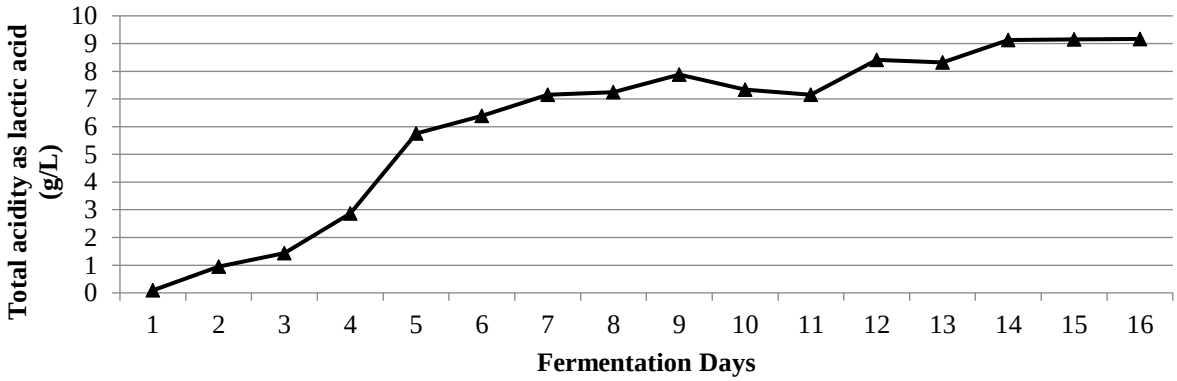

Figure 3. Total acidity during fermentation

Samples were filtered and stored at 4°C for further analyses. Then, fermented salgam samples were freeze-dried and converted to powder form. Freeze-drying mainly uses the sublimation and desorption phenomenon to remove most of the water in a product. It generally takes place at atmospheric pressure in an inert gas atmosphere, however most of the freeze-drying operations are carried out under vacuum (Di Matteo *et al.,* 2003; Ratti, 2012). Sublimation is the transformation of ice directly into a gas without passing through a liquid phase (Welti-Chanes *et al.,* 2005). Freeze-drying is a very good drying method for obtaining final products at the high quality. An important point for freeze-dried foods is that freeze-dried powder should be

readily soluble in water. For the determination of that powder salgam was reconstituted by water at different ratios (1.5, 2.5, 3, 3.2 and 3.5 % powder). Then total acidity and pH analyses were conducted on the reconstituted salgam samples, because acidity of salgam is one of the most important criteria affecting the choice of the consumer. Titratable acidity should be at least 6 g/L as lactic acid and pH should be maximum 3.8 according to Turkish Salgam Standart TS 11149 (TSE, 2016). Total acidity values of the powder at 3.2 and 3.5 % were determined as 6.3 g/L ve 7.86 g/L, respectively. These results showed that powder should be at least 3.2 % to meet the requirements of the TS 11149 salgam standard. Therefore, 3.2% powder was chosen for the production of salgam and analyses were conducted after adding of water on powder at the ratio 3.2 %. Physicochemical and sensorial characteristics of reconstituted salgam sample that was prepared by instant powder salgam at the ratio of 3.2 % was investigated by using fermented salgam as control. Results of physicochemical properties are shown in Table 1.

The principal end product of salgam fermentation is lactic acid which accounts for the majority of total acidity. Its concentration ranges from 3.92 to 8.17 g/L in different salgam samples (Canbas and Deryaoglu, 1993; Ozturk, 2009; Tanguler and Erten, 2012a, b; Tanguler *et al.,* 2014a, b; Agirman, 2014; Tangüler *et al.,* 2015). Total titratable acidity should be at least 6 g/L as lactic acid according to Turkish Salgam Standart TS 11149 (TSE, 2016). Lactic acid formed during fermentation helps to preserve the beverage and enhances taste and aroma (Erten and Tangüler, 2016). Reconstituted salgam had the requirements of the TS 11149 salgam standard for the values of pH, total acidity and salt content as 3.70, 6.30 g/L and 1.57%, respectively.

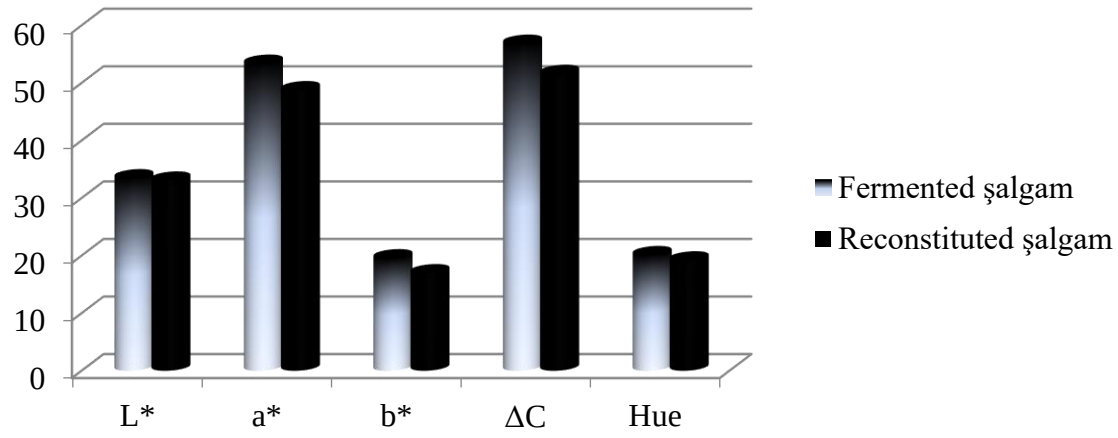

Figure 4. Colour parameters for fermented and reconstituted salgam

Colour is an important quality criteria for salgam. Red colour of shalgam derives from anthocyanin compounds that are present in black carrots. Main phenolic compounds in salgam are anthocyanins (Erten and Tanguler, 2016). During the fermentation, these colour compounds, anthocyanins, in the black carrot are released into the salgam (Erten and Tanguler, 2016). But it was reported that high temperature is an important factor responsible for anthocyanin loss (Turker *et al.,* 2004) and it was reported that storage at lower temperatures prevents the

anthocyanine losses (Turker *et al.,* 2004; Tokgöz *et al.,* 2010; Kelebek *et al.,* 2011; Zhang *et al.,* 2015). In the present study, freeze-drying did not cause to the anthocyanin loss. Total phenol and anthocyanin contents of the fermented and reconstituted salgam samples were 268.95 and 242.05 mg/L as gallic acid and 284.8 and 296.5 mg/L as cyanidine 3 glucoside, respectively. There was not any significant difference between total phenol and anthocyanin contents of the fermented and reconstituted salgam samples ($p>0.05$). It was reported in the study of Lutz and others (2015), dehydrated products can be good sources of phenolic compounds since total phenolic contents were higher in some vegetables and fruits than the fresh counterparts (Lutz *et al.,* 2015).Colour characterization of the samples were done by using Hunter Lab in the present study. Chromatic values L*, a* and b* were determined and Chroma (ΔC) and hue angle (Hue) values were calculated based on the equations to define the colour of salgam samples. L*, a*, b* and calculated Hue and Chroma values are shown in Table 1. Results of the present study showed that L*, a*, b* values of the reconstituted salgam was close to the fermented salgam (Figure 4). L*, a*, b* values are calculated as 33.4 and 33.0, 53.3 and 48.7, 19.5 and 16.9 for fermented and reconstituted salgam samples, respectively. Lightness of the colour was defined by L*parameter and positive a* and b* values indicate red and yellow, respectively (Gould, 1977; Hunter and Harold, 1987; Wojdylo *et al.,* 2009). Results of the present study showed that salgam samples have a specific red colour. The differences between the L*,a*, b* values were not significant ($p>0.05$).

Turbidity levels of fermented and reconstituted salgam samples were determined as 292 and 477.5 NTU (nephelometric turbidity unit), respectively. As it can be seen turbidity of reconstituted salgam was higher than fermented salgam but the difference was not significant ($p>0.05$). During the fermentation of salgam, colour and other compounds are diffused to the aqueous phase and then salgam is filtered and packagad. On the other hand, when powder salgam is reconstituted, turbidity increased due to the presence of suspended solid of powder.

Sensorial evaluation results are shown in Figure 5. For the sensory analysis of salgam samples, 11 panelists evaluated samples by using a 10-point hedonic scale ranging from 1 (dislike extremely) to 10 (like extremely) and also ranked each sample from most preferred to least preferred in terms of colour, odour and flavour, taste, acidity (sourness) and overall sensory impression. Instant salgam sample was also preferred by panelists. It had got the scores slightly less than fermented salgam from the colour and taste points of view. Mean scores of fermented and instant salgam on the hedonic scale was 9.57 and 9.14 for colour and 8.5 and 7.9 for taste, respectively. From the odour and flavour, acidity (sourness) and overall sensory impression points of view, samples had closer results. Mean scores of fermented and instant salgam were 8.64 and 7.14 for odour and flavour, 8.07 and 6.43 for acidity (sourness) and 8.99 and 7.21 for overall sensory impression. Acidity can be increased by increasing the powder ratio in reconstituted salgam and as a result of that it is possible to increase sensorial evaluation for that property. As it can be seen, fermented salgam had higher scores when it was compared to instant salgam. However, results of sensory evaluation showed that instant salgam is a product that can be preferable by consumers.

Table 1. Physicochemical properties of fermented and reconstituted salgam samples

Physicochemical property		**Fermented salgam**	**Reconstituted**	**Importance**	**TS11149**
Solid matter (%)		3.08 ± 3.04	2.66 ± 0.77	p>0.05*	≥2.8
Salt (%)		1.67 ± 0.007[a]	1.57 ± 0.07[b]	p<0.05**	≤1.7
Total acidity[1] (g/L)		9.16 ± 0.19[a]	6.30 ± 0.04[b]	p<0.05**	≥6.0
pH		3.62 ± 0.02[b]	3.70 ± 0.00[a]	p<0.05**	≤3.8
Total anthocyanin content[2] (mg/L)		284.8 ± 25.03	296.5 ± 31,67	p>0.05*	-
Total phenol content[3] (mg/L)		268.95 ± 13.22	242.05 ± 34.71	p>0.05*	-
Turbidity (NTU[4])		292 ± 69.29	477.50 ± 258.09	p>0.05*	-
Colour	L*	33.4 ± 0.03	33.0 ± 0.22	p>0.05*	-
	a*	53.3 ± 0.58	48.7 ± 0.85	p>0.05*	-
	b*	19.5 ± 0.18	16.9 ± 0.16	p>0.05*	-
	Chroma	56.77 ± 0.61	51.54 ± 0.86	p>0.05*	-
	Hue	20.05 ± 0.29	19.13 ± 1.43	p>0.05*	-

[1]as lactic acid, [2]as cyanidin 3-glucoside, [3]as gallic acid, [4]nephelometric turbidity unit, * not significant at 5 % by LSD (lEast significance difference), **significant at 5 % by LSD (least significance difference)

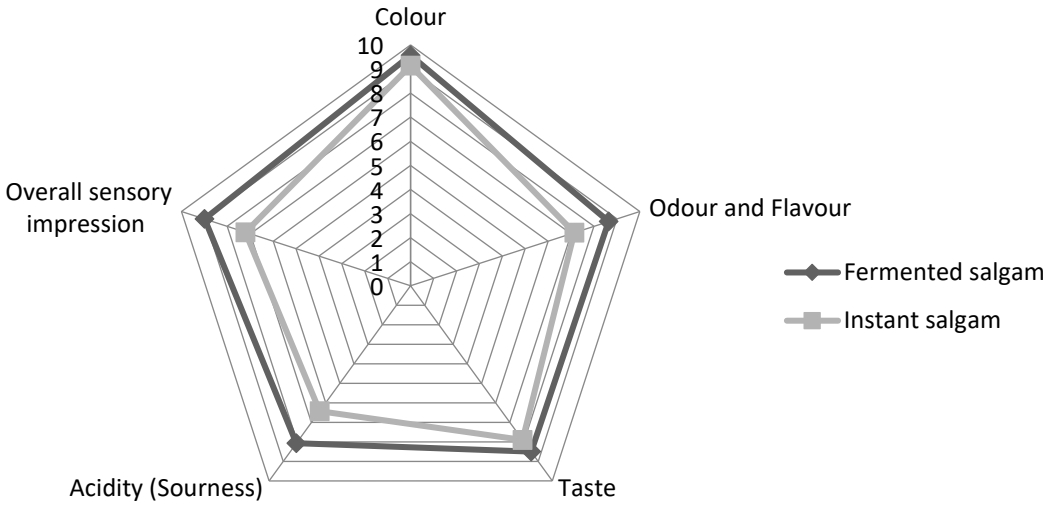

Figure 5. Sensorial evaluation by spider-plot

4. Conclusions

In the present study, fermented salgam was produced traditionally and then water of salgam was removed by vacuum freeze-drying technique and powder form of salgam was obtained. Results showed that reconstituted salgam produced by using 3.2 % powder met the requirements of the salgam standard. pH, total acidity and salt content of the the instant formof the product was close to fermented salgam. Also, sensorial evaluation results showed that colour and taste of the salgam samples were almost same for both of them.

Vacuum freeze-drying of fermented salgam results in a final product with good sensorial properties. Freeze-drying of salgam can provide convenience in transportation and handling as a result of the reduced volume and weight. Moreover, powder form has longer shelf life when it is compared with the fermented salgam. Another advantage of powder form is reducing packaging costs. On the other hand, there is a need for freeze-drier with high operational costs. In conclusion, it is the first study on the production of instant

salgam powder by freeze-drying technique and results of present study showed that instant salgam was preferred by paneslists from the sensorial point of view and possible to accepted by consumers as the ready-to-use product. However, further studies need to be done for better knowledge.

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BEHAVIOR OF *Escherichia coli* O157 DURING THE MANUFACTURE AND RIPENING OF A TRADITIONAL SICILIAN RAW EWES' MILK CHEESE

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ABSTRACT

To evaluate the ability of *Escherichia coli* O157 to growth and survival on wooden vat surface during the manufacture process and subsequent ripening of traditional raw milk cheese, *Pecorino Siciliano* PDO cheese (protected denomination of origin) - made from raw ewes' milk - was used as a model system. Two wooden vats were activated with deproteinized whey in a dairy pilot plant. One vat was used for control production and one for experimental production performed after a massive contamination of milk with a multi-strain combination of *E. coli* O157. Cheese making processes were monitored from wooden vats surface to ripened cheese by performing microbiological and chemico-physical analysis. The results of pH, activity water (a_w) and LAB concentration showed similar trends in both productions. In particular, pH and a_w decreased during the production and ripening while LAB count increased. The specific investigation of *E. coli* O157 in experimental production showed the inability of this pathogen to adhere and survive on wooden vat biofilms: this may be due to the acidity and the bacteriocin generated by LAB that represented efficient barriers to their adhesion. The level of *E. coli* O157 in cheese decreased progressively but their presence was detected up to 90 days of ripening showing the ability of this bacteria to survive at low values of pH (5.20) and a_w (0.920). *Escherichia coli* O157 was never detected at 120 days of ripening. These results confirmed that the ripening and the synergistic effect among acid condition generated by LAB and the low a_w are able to delete this pathogen from raw ewes' milk cheeses.

1. Introduction

Escherichia coli O157 represent one of the most important food pathogen. It is frequently found in a wide range of food products, including raw and pasteurized milk and milk derived products (Heuvelink *et al.*, 1998; McIntyre *et al.*, 2002), such as cheeses (Honish *et al.*, 2005; Strachan *et al.*, 2005). Since its discovery in 1983 (Riley *et al.*, 1983), *E. coli* O157 has been considered an important foodborne pathogen that causes hemorrhagic colitis, hemolytic uremic syndrome, thrombotic thrombocytopenic purpura, and can even lead to death (Doyle and Cliver, 1990). *E. coli* O157 can survive at low temperatures under acidic conditions, as well as at high salt concentration, and the infectious dose is relatively low (Glass *et al.*, 1992; Coia, 1998; Park *et al.*, 1999). The main natural reservoir of this microorganism is represented by the gastrointestinal tract of ruminants, especially dairy cows, followed by sheep and pigs (Zhao *et al.*, 1995). The infection can be transmitted from animals to

human beings by different routes, including direct and indirect contact (Parry *et al.*, 1995; Trevena *et al.*, 1996; Coia, 1998; Tozzi *et al.*, 2001). The presence of these bacteria in dairy products is usually associated with inadequate hygiene practices as a consequence of fecal contamination (Alemdar and Ağaoğlu, 2016). After hamburgers, raw milk has been identified as the most important vehicle for the transmission of *E. coli* O157 (Reitsma and Henning, 1996) while, between the dairy products, raw milk cheese is known to be the most frequently contaminated (Bielaszewska *et al.*, 1997; EFSA, 2013).

All traditional Sicilian cheeses that enjoy protected designation of origin (PDO), as *Pecorino Siciliano*, *Piacentinu Ennese*, *Ragusano* and *Vastedda della valle del Belìce*, are manufactured in small size farms with raw milk from animals of indigenous breeds, without the addition of starter cultures and using wooden equipment (Horne *et al.*, 2005; Gaglio *et al.*, 2014, 2016; Carpino *et al.*, 2017). Recently, some studies showed the ability of indigenous lactic acid bacteria (LAB), commonly present in raw material (mainly milk and animal rennet) and on biofilms of the wooden vat surfaces, to inhibit the growth of dairy pathogenic bacteria such as *Listeria monocitogenes* (Macaluso *et al.*, 2016; Scatassa *et al.*, 2015, 2017). However, there is an increasing concern that these cheeses, produced with raw milk and wooden equipment, may pose a threat to consumer safety by transmitting pathogens such as *E. coli* O157 (Vernozy-Rozand *et al.*, 2005). Between traditional Sicilian cheeses, *Pecorino Siciliano* represents the principal dairy production throughout the regional area (Guarcello *et al.*, 2016). *Pecorino Siciliano* is a semi-hard cheese manufactured following traditional techniques according to the production protocol (GURI n. 295/1955).

There are many studies regarding the microbiological, chemical and sensorial properties of *Pecorino Siciliano* cheese (Settanni *et al.*, 2013, Guarcello *et al.*, 2016) and on the microbial characterization of the wooden vat used for its production (Scatassa *et al.*, 2015). However, the behavior of *E. coli* O157 in *Pecorino Siciliano* and the ability of this bacteria to adhere and survive on wooden vat surface used for cheese production are not known.

In this context, the present work was aimed to evaluate the potential growth and survival of *E. coli* O157 on wooden vat surface, during manufacture process and subsequent ripening of *Pecorino Siciliano* cheese.

2. Materials and methods

2.1. Wooden vat activation

Two Calabrian chestnut wooden vats with a 100-liter volume were purchased from a local artisanal producer. Wooden vat activation was made as reported by Cruciata *et al.* (2018). Briefly, the two vats were treated daily with hot water (75 to 80°C) for 30 days in order to remove the tannin components from wood. before biofilm activation. After that, the LAB biofilms formation on the wooden surfaces was performed with hot deproteinized whey (about 80°C), that is the residual whey from *ricotta* cheese production, left in contact with the vats for 24 h. The treatment with hot deproteinized whey was repeated for seven consecutive days. Furthermore, after this treatment in both vats a step of cheese production was carried out with only the aim of promoting LAB biofilms formation on wooden vat surfaces.

One vat was used as control production (CP) and one as experimental production (EP) in order to investigate the ability of *E. coli* O157 to grow and survive on the wooden vat surfaces, during manufacture process and subsequent ripening of cheeses.

2.2. Strain and growth condition

Three strains of *E. coli* O157 (EF 348; EF 358 and EF 390) obtained from the culture collection of the European Union Reference Laboratory for *Escherichia coli* (Roma, Italy) was propagated in Brain Heart Infusion (BHI) broth (Oxoid) at 37°C for 18-24 h. For each strain was prepared a subcultures incubated as described above and after growth were mixed in order to obtain a multi-stain inoculum.

Before milk inoculation the multi-strain combination of *E. coli* O157 was maintained under given storage conditions (4°C) for 24 h according to EURL *Lm* Technical Guidance Document (2014). The last step was applied in order to remove the stress condition of the multi-strain combination of *E. coli* O157 during the inoculation in refrigerated raw ewes' milk.

2.3. Cheese production sample collection

Cheese production was carried out under controlled conditions in a dairy pilot plant (Istituto Zooprofilattico Sperimentale della Sicilia A. Mirri, Palermo, Italy) using POLYFOOD mod. SI-050 (INVENTAGRI™, Modena, Italy).

Refrigerated raw ewes' milk (180 liters) was divided in two aliquots (90 liters each) representing two different trials (CP and EP). EP was inoculated with multi-strain combination of *E. coli* O157 at a final concentration of approximately 10^5 CFU/mL (verified by plate counting) to simulate massive contamination, while CP was supplemented with the same volume of Ringer's solution without bacteria. Both aliquots of raw ewes' milk were heated at 38±1°C and transferred in the respective wooden vats. Subsequently, both bulks were then subjected to traditional cheese making provided by the production protocol of *Pecorino Siciliano* PDO cheese (GURI, 1955) ripened for 4 months in a storage chamber at 13°C and 80% of relative humidity (RH) as reported in figure 1. Cheese trials were carried out in duplicate in two consecutive weeks in Spring 2016.

The following samples were collected during each cheese production: samples from vat surface, bulk milk, bulk milk with inoculum, curds just after curdling and cheese at 0, 30, 60, 90 and 120 days of ripening. Wooden vat surfaces (100 cm^2) were sampled, just before starting cheese production, as reported by Didienne *et al.* (2012) using UV-treated paper squares positioned halfway up the side and on the bottom. Sampling points and analyses performed during cheese production are reported in table 1.

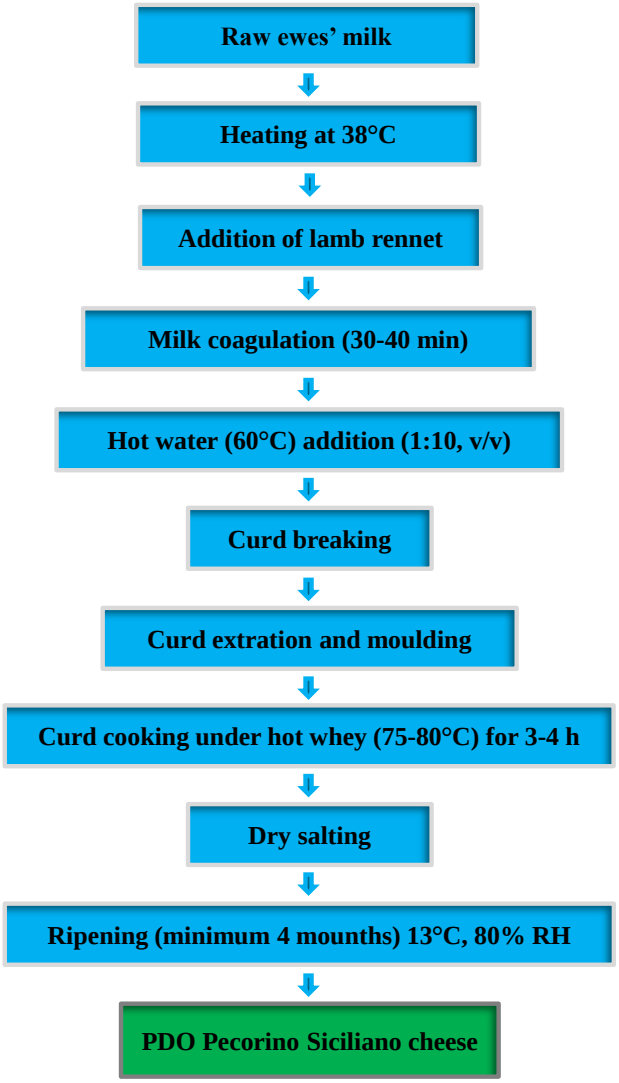


Figure 1. Flow diagram of PDO *Pecorino Siciliano* cheese production.

2.4. Microbiological and chemico-physical analyses

Cell suspensions of the wooden vat surface and milk samples were subjected to decimal serial dilutions in Ringer's solution, while curd and cheese samples were first homogenized in Ringer's solution (1:10) in a stomacher (400 Circulator Bags; Seward, AK, USA) for 10 min at 260 rpm, and then serially diluted. Inoculation, cultivation, and incubation of the different microbial groups were as follows: presumptive rod LAB were grown on MRS agar, acidified to pH 5.4 with lactic acid (5 mol/L), incubated anaerobically for 72 h at 37°C; enumeration of *E. coli* O157 was carried

out on CHROMagar™ O157 incubated aerobically for 24 h at 37°C while the detection by the method AFNOR BIO 12/25-05/09.

Temperatures during production and ripening processes were monitored by data logger (Thermo button IBee 22T– Plug and Track by proges Plus – France), pH of milk, curd and cheeses samples were measured by a portable pH meter HI3220-02 (Hanna Instruments, Woonsocket, Rhode Island, USA). Water activity (a_w) was determined following ISO 21807:2004 method using an activity-meter instrument (HygroLab, Rotronic, Bassersdorf, Switzerland). Microbiological and chemico-physical analyses were carried out in triplicate.

Table 1. Sampling points[a] and analyses performed during cheese production.

Analyses	Sampling points											
	WV			BM		BMI		CG			Ch (t_0,t_{30},t_{60},t_{90}, t_{120})	
	CP	EP						CP	EP		CP	EP
pH				■		■		■	■		■	■
a_w											■	■
LAB counts	■	■		■		■		■	■		■	■
E. coli O157	■	■		■		■		■	■		■	■

[a] Three samples were analysed for each production.

Abbreviations: WV, wooden vat; BM, bulk milk; BMI, bulk milk after inoculum with *E. coli* O157; CG, curds just after curdling; Ch, cheese at 0, 30, 60, 90 and 120 days of ripening; CP, control; EP, experimental; a_w, water activity.

2.5. Statistical analyses

Microbiological and chemico-physical data were subjected to one-way variance analysis (ANOVA). Pair comparison of treatment means was achieved by Tukey's test at $P < 0.05$. Statistical analysis was conducted using XLStat software version 7.5.2 for excel (Addinsoft, New York, USA).

3. Results and discussions

In this study, we evaluated the ability to growth and survival of *E. coli* O157 on wooden vat surface, during the manufacture process and subsequent ripening of *Pecorino Siciliano* cheeses. The cheese making was conducted at pilot-scale level in standard conditions in order to keep all process variables.

The results of microbiological and chemico-physical analysis of samples collected during control production (CP) and experimental production (EP) are reported in table 2. The pH decreased during coagulation and during all the ripening days (from around 6.80 to 5.20) as well the a_w (from around 0.980 to 0.910) whereas the LAB count increased showing a similar trends in both production. Statistical differences according to Tukey's test were found between the samples collected during each production in terms of microbial loads, pH and a_w.

During the two productions both wooden vat surfaces CP and EP after milk coagulation hosted high numbers of LAB and their level were about 5 log CFU/cm^2 as reported in a recent investigation on surfaces used for traditional Sicilian cheeses production (Scatassa *et al.*, 2015). The bulk milk and the inoculated bulk milk showed LAB count of 5.86 and 5.21 log CFU/mL, respectively. These results confirmed that the wooden equipment and the milk represent the main source of desirable LAB might act as cultures useful in cheese making (Beresford *et al.*, 2001; Di Grigoli *et al.*, 2015). The kinetics of LAB grow of both CP and EP samples were similar, showing a consistent increase in concentration after the coagulation reaching in cheeses during the entire periods of ripening values higher of 8 Log CFU/g. Similar results were previously reported by Settanni *et al.* (2013) in PDO *Pecorino Siciliano* after 5 month of ripening. The specific investigation of *E. coli* O157 on

wooden vat surfaces did not generate any colony in both wooden vats. This may be due to the presence of LAB biofiolms, the acidic conditions generated by the development of LAB in whey and the production of bacteriocins (Lortal *et al.*, 2009). Relatively to cheeses, previous studies showed that *E. coli* O157 may survive during manufacture and ripening and the number of this bacteria increases during storage (Maher *et al.*, 2001). In the present study, *E. coli* O157 was found in milk used in experimental production at almost the same levels of inoculation (4.69 CFU/mL) and his concentration increased in cheese after production (Cht_0) reaching a value of 5.20 CFU/g. After that, the counts of *E. coli* O157 decreased continuously and it was never found at 120 days of ripening. Inactivation of *E. coli* O157 at 120 days of ripening is mainly due to a synergistic effect among acid condition generated by LAB and to the low a_w. The last parameter, represent the most important factor that influences the microbial growth in foods as reported by Hamad (2012).

The results of the present study showed that in experimental *Pecorino Siciliano* production the *E. coli* O157 was able to survive up to 90 days of ripening with a_w value equal to 0.920 while this bacteria was totally deleted at 120 days with a_w value equal to 0.910.

Table 2. Microbial loads[a] and chemico-physical analysis of samples collected during experimental *Pecorino Siciliano* cheese making.

Samples	**Parameters**			
	pH	**a_w**	**LAB**	***E. coli* O157**
Control production				
WV	n.e.	n.e.	4.71 ± 0.12^{a}	n.d.
BM	6.73 ± 0.01^{b}	n.e.	5.86 ± 0.02^{b}	n.d.
CG	6.34 ± 0.01^{b}	n.e.	7.33 ± 0.04^{c}	n.d.
Cht_0	5.33 ± 0.02^{a}	0.980 ± 0.01^{b}	8.07 ± 0.09^{d}	n.d.
Cht_{30}	5.20 ± 0.01^{a}	0.945 ± 0.02^{ab}	8.94 ± 0.02^{f}	n.d.
Cht_{60}	5.22 ± 0.03^{a}	0.937 ± 0.02^{a}	8.79 ± 0.03^{ef}	n.d.
Cht_{90}	5.25 ± 0.01^{a}	0.928 ± 0.02^{a}	8.57 ± 0.03^{e}	n.d.
Cht_{120}	5.26 ± 0.01^{a}	0.913 ± 0.02^{a}	8.17 ± 0.07^{d}	n.d.
Statistical significance	*	*	***	
Experimental production				
WV	n.e.	n.e.	4.82 ± 0.21^{a}	n.d.
BMI	6.80 ± 0.01^{b}	n.e.	5.21 ± 0.12^{b}	4.69 ± 0.27^{c}
CG	6.54 ± 0.04^{b}	n.e.	6.04 ± 0.05^{c}	4.77 ± 0.01^{c}
Cht_0	5.34 ± 0.05^{a}	0.980 ± 0.03^{c}	8.13 ± 0.07^{d}	5.20 ± 0.11^{d}
Cht_{30}	5.17 ± 0.02^{a}	0.963 ± 0.02^{bc}	8.91 ± 0.03^{e}	4.55 ± 0.12^{c}
Cht_{60}	5.24 ± 0.01^{a}	0.936 ± 0.02^{a}	8.33 ± 0.06^{d}	2.89 ± 0.11^{b}
Cht_{90}	5.26 ± 0.03^{a}	0.920 ± 0.01^{a}	8.30 ± 0.04^{d}	1.30 ± 0.40^{a}
Cht_{120}	5.28 ± 0.02^{a}	0.907 ± 0.04^{a}	8.61 ± 0.03^{e}	n.d.
Statistical significance	**	*	***	***

[a] Units are Log CFU/cm^2 for wooden vat surface; Log CFU/mL for milk samples; log CFU/g for curds and cheeses.
Data within a column followed by the same letter are not significantly different according to Tukey's test.
P value: *P < 0.05; **P < 0.01; ***P < 0.001.
Abbreviations: WV, wooden vat; BM, bulk milk; BMI, bulk milk after inoculum with *E. coli* O157; CG, curds just after curdling; Ch, cheese at 0, 30, 60, 90 and 120 days of ripening; a_w, water activity; LAB, lactic acid bacteria; n.e., not evaluated; n.d., not detected in 100 cm^2 of wooden vat surfaces, 25 mL or g of milk, curd and cheese respectively.
Abbreviations: n.e., not evaluated; n.d. not detected.

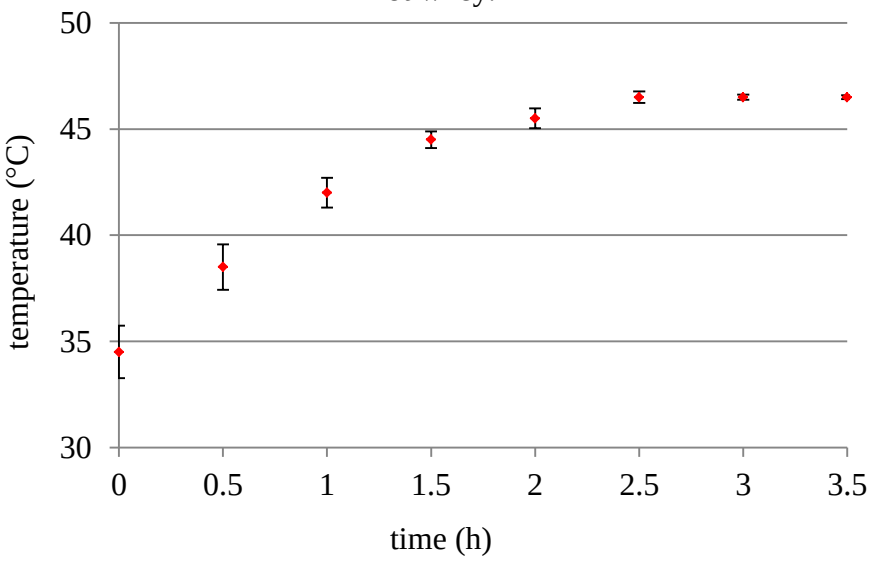
Table 3. Changes of temperatures during experimental *Pecorino Siciliano* cheese making.

Step	Time	Temperatures
Raw ewes' milk	n.e.	38±1°C
Addition of lamb	n.e.	36°C
Milk coagulation	40-45 min	34±1°C
Hot water addition	n.e.	65°C
Curd breaking	15 min	37±1°C
Curd moulding	10 min	33 ±1°C
Curd cooking	4 h	40±7°C*
Dry salting	n.e.	19±1°C
Ripening	120 days	13°C

*referred to the temperature monitoring in the heart of cheese.
Abbreviations: n.e., not evaluated.

Results of temperature monitoring during experimental *Pecorino Siciliano* cheese making are reported in table 3. The multi-strain combination of *E. coli* O157 used in this study showed the ability to survive at all heat treatment applied during cheese production and in particular at the higher temperature (46.5°C) reached in the heart of cheese after 2.5 h of curd cooking under hot whey as reported in figure 2.

Figure 2. Temperatures recorded in the heart of *Pecorino Siciliano* cheese during the cooking under hot whey.



4. Conclusions

This study is the first report on the ability of *E. coli* O157 to grow and survive on the wooden vat surfaces, during the manufacture process and subsequent ripening of *Pecorino Siciliano* cheese. The results of the present work confirmed the inability of this pathogen to adhere or to survive on wooden vat biofilms. However, the massive contamination of the raw ewes' milk determined the survival in *Pecorino Siciliano* cheese of *E. coli* O157 at least 90 days of ripening. *E. coli* O157 was never detected in experimental cheese production at 120 days of ripening showing that the inactivation of this bacteria is mainly due to a synergistic effect among acid condition generated by LAB and the low a_w. Therefore, during the production of *Pecorino Siciliano* the ripening represents the most important step in order to delete this dairy pathogen.

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INTEGRATED NUTRIENT MANAGEMENT: SOIL HEALTH, NITRATE TOXICITY AND TUBER QUALITY IN POTATO (*SOLANUM TUBEROSUM* L.) GROWN IN SUBTROPICAL PUNJAB

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ABSTRACT

Potato (*Solanum tuberosum* L.) is a fertilizer intensive crop and require 160, 100 and 100 kg per hectare of N, P and K, respectively. Application of recommended dose of nitrogen through urea has been reported to increase nitrate content of tubers which has increased risk towards cancer, so increasing yield on the cost of human health is not acceptable. The present research emphasises the role of biofertilizers in soil and plant nutrient status, reduction of nitrate toxicity in potato tubers and improvement of tuber quality. The results of experiment confirm the buffering nature of biofertilizers to maintain soil pH upto 7.56 in T_7 and 7.70 in T_8, improvement of soil organic matter upto 5.52% in T_8, highest total nitrogen (78.83 Kg/ha) in T_8, highest available P (77.57 Kg/ha) in T_2 while highest available K (202.03 Kg/ha) in T_6. The highest leaf N, P, K and chlorophyll (48.77, 5.833, 86.626 and 20.951 mg/ml, respectively) was reported in T_8 (50% RDF + PSB + *Azotobacter* + VAM + Mustard cake). Tuber quality was improved in term of mineral (Ca, Fe and Zn) content and reduced nitrate content when 50% and 25% of urea was replaced with Azotobacter or vermicompost and integrated with PSB (Phosphate Solubilizing Bacteria) or VAM (Vesicular Arbascular Mycorhhizal) fungi without decreasing the tuber yield.

1. Introduction

Potato (*Solanum tuberosum* L.), belongs to family solanaceae, is major vegetable crop grown throughout the tropical, subtropical and temperate world for vegetable production or as staple crop or for true seed production (Lutaladio and Castaldi, 2009). It is also considered as a major staple crop because per hectare dry matter and protein production of potato is more than the cereals like wheat, rice and maize (Singh and Rai, 2011). Potato tubers have high nutritional value and energy content; it has very high economic advantages which will be suitable for developing economy (Van Gijssel, 2005; McGregor, 2007). The problem of malnutrition and under nutrition can be easily solved if potato is accepted in our country as a major food and not merely as a vegetable in our country. India ranks third position in production of potatoes following China and Russia and accounts of 41555 thousand MT to area of 1973 thousand hectares and productivity 21% per MT per hectare (Indian Horticulture Database, 2014).

Potato crop needs a perfect balanced fertilization or else the development and growth

of the crop will be poor and ultimately will affect the yield and quality of tubers. Nitrogen (N) and phosphorus (P) the major nutrients in potato production along with potassium (P). The abundance and low cost of N fertilizer has encouraged the farmers to use the high fertilization rates in attempts to obtain maximum tuber yields. Plant take nitrogen in form of nitrate and ammonium ion and utilize them for synthesis of nucleic acids, proteins, chlorophyll and many nitrogen containing compounds (Lea and Ireland, 1999). The balance between absorption, translocation and assimilation of nitrates in the plant body is essential and if this balance is disturbed most of the nitrate is concentrated to the root and tubers (Cash *et al.*, 2002). High level of Nitrate in potato tuber; (>67 ppm) leads to high nitrate concentration in human body, which is further reduced to nitrite. High nitrite concentration may cause meth-hemoglobinemia or may combine with amino acids to form a potential carcinogenic substance called nitrosamine (Breimer, 1980). The excess application of nitrogen not only damages the crop quality and environment but also represents unnecessary economic expenditure of the farmers. The high cost of chemical fertilizers along with the related ecological and health hazards necessitate finding out the alternate nutrient sources to sustain the crop yield without any adverse effect on soil and environment.

The bio-fertilizers such as *Azotobacter, and* phosphobacteria (PSB) have been recognized as important inputs for improving soil health and fertility for optimum crop production (Dobereiner, 1997). However, their effects vary with crops, soil and environmental conditions. Tyagi, *et al.* (1999) had reported that bio-fertilizer reduces consumption of inorganic fertilizers by 20 - 50 per cent and can improve the crop yield by 10 - 20 per cent. Organic matter can bind different minerals like Mg and K in their colloids form of clay and humus, to facilitate formation of stable soil aggregates and improve porosity. Microbial biomass, sources of microbes present in biofertilizers add organic matter to soil so can improve soil condition (Perucci, 1990) and can be better option than FYM for improving potato tuber yield (Farag *et al.,* 2013; Narayan *et al.,* 2013). Considering the significance of balanced fertilization for economic yield of potato the present study put emphasis on bio-fertilizer application in potato to improve soil health, plant nutrients and nutritive value to potato tuber.

2. Materials and methods

The present study is based on the investigation involved a field experiment on potato plant conducted during Rabi season of the year 2015-16 at the Agricultural Research Farm of the School of Agriculture, Lovely Professional University, Jalandhar, Punjab (India). The experimental site is characterized as "Central Plain Zone (PB-3)" of Punjab and it is located at 31º 15' N latitude and 75^0 41' E longitudes at an elevation of 245 m above mean sea level. Investigation was carried out in area of 500m^2which was divided into 24 plots with area of 20 m^2 through the proper layout plan.

There were eight treatments each with three replications. The treatments used were T_1 (100% RDF-Recommended Dose of Fertilizers), T_2(50% RDF + PSB+ VAM), T_3(50% RDF + PSB+ Mustard cake), T_4 (50% RDF + PSB+ *Azotobacter*), T_5 (50% RDF + PSB+ VAM+ Mustard cake), T_6 (50% RDF + PSB+ VAM + *Azotobacter*), T_7 (50% RDF + PSB+ *Azotobacter*+ Mustard cake) and T_8 (50% RDF + PSB+ *Azotobacter*+ VAM+ Mustard cake).

Sprouted tubers with uniform size 30- 40 g of Kufri Jyoti variety were planted at spacing of 25x60 cm. After planting, ridges were made to cover the tubers. Planting was done on 22nd October 2015.

2.1. Observations Recorded

2.1.1. Soil nutrient status

The soil samples were collected with the help of spade before and after the experiment. The soil from 4 different places was randomly collected from each plot and mixed together to get representative samples. The soil samples

were carried out the laboratory and dried in the oven at the temperature of 105^0C till the constant weight was obtained. The soil samples were used for analysis of soil pH, EC, organic matter content, available nitrogen, available phosphorus and available potassium. Soil pH was obtained with the help of digital pH meter using 1:2.5 soil-water suspensions as advocated by Metson (1956). Electrical conductivity of soil was measured by EC meter by weighing 25 g of soil and adding 50ml of distilled water to it, stirring it continuously 4-5 times and the leave it overnight and then measures EC with EC meter. Organic matter was estimated by Walkley and Black (1934) "Rapid titration method"; total nitrogen was estimated by alkaline potassium permagnate ($KMnO_4$) method; available phosphorus was estimated by Olsen's method and available potassium was estimated by flame photometer with the use of saturation extract of soil as described by Baruah and Banthakur (1998).

2.1.2. Potato leaf nutrient status

The nutrient content of potato leaves was estimated by sampling leaves at full maturity i.e. in month of January 2016. Thirty leaves from third and fourth pairs from the apex were collected from five shoots. The sampled leaves were washed thoroughly with tap water and dipped in 0.1 N HCl, distilled water, and then oven dried at 60^0C for constant weight. The digestion of the plant materials for various nutrients was done in di-acid mixture (AR graded, distilled H_2SO_4 and AR graded per-chloric acid) in 3:1 ratio and estimation was done as discussed earlier.

2.1.3. Chlorophyll content of leaves

Concentration of chlorophyll in photosynthesis tissue of plants i.e. leaves was done by the procedure defined by Arnon (1949) and five leaves were collected from tagged plants and grounded in solution mixture containing 0.1 N ammonium hydroxide and acetone in 1:9 ratio by volume .The mixture was centrifuged and supernatant was diluted to concentration so that the absorbance at wavelength 663 nm (A_{663}) and 645 nm (A_{645}) ranged between 0.2 and 0.8.the absorbance at above wavelength was measured and Ch-a, Ch-b and total chlorophyll was calculated by following formulae and was expressed in mg/ml:

$$\text{Ch-A} = 12.7\ A_{663} - 2.69\ A_{645}$$

$$\text{Ch-B} = 22.9\ A_{663} - 4.68\ A_{663}$$

$$\text{Total chlorophyll} = \text{Ch-A} + \text{Ch-B}$$

2.1.4. Tuber quality analysis

Twenty grams of tubers from each replication was taken, dried at about 60% of moisture and ground with the help of grinder. The ground material was further dried for 2hrs at 60^0C and weight of samples was taken. Nitrate content was determined by the phenoldisulferic acid method using water extract of the plant tissue (Johnson and Ulrich, 1959). A 2% (w/v) solution of trichloroacetic acid (TCA) was prepared in distilled water. Samples (0.5g) of dried tuber were weighed into 100 ml Kjeldhahl flasks containing a glass bead and was digested with 5ml of HNO_3 followed by cooling and further digestion in 5 ml mixture of HNO_3 and $HClO_4$ in 3:1.40 ml of distilled and demineralised water was added and brought to boiling to ensure complete digestion. Samples were cooled, diluted to 50 ml and filtered through Whatman No. 50 paper. Atomic absorption spectrophotometer was used to determine metal concentration. Zn and Fe were determined directly on the filtrates while filtrate was diluted 2.5 times with a solution containing 15 La and 5% HCL.

3. Results and discussion

3.1. Soil nutrient status

3.1.1. Soil pH

Application of biofertilizers had significantly improved soil pH (Table 1) in comparison to soil at the time of planting tubers. Lowest soil pH (7.56) was reported in T_7 (50% RDF + PSB+ *Azotobacter*+ Mustard cake) followed by 7.70 in T_8 (50% RDF + PSB+ *Azotobacter*+ VAM+ Mustard cake), 7.90 in T_6 (50% RDF + PSB+ VAM +

Azotobacter) and 7.93 in T_5 (50% RDF + PSB+ VAM+ Mustard cake). Thus, highest reduction in soil pH was reported in T_7, followed by T_8. The improvement of soil pH due to application of biofertilizers like *Azotobacter*, VAM, PSB and mustard cake in various treatments applied during potato cultivation might be production of organic acids due to decomposition of organic matter present in soil and added by vermicompost and the applied biofertilizers. The released acids were capable to neutralize alkalinity of soil. The present finding is in conformity with the findings of Ansari (2008) who proposed application of vermicompost has reduced pH from 9.51 to 8.41 (lowest value) in potato, spinach and turnip growing soil.

Table 1. Soil nutrient status after biofertilizers application in potato

Treatments	Soil pH	Soil EC (dS/m)	Soil O.M. (%)	Total Nitrogen (Kg/ha)	Available Phosphorus (Kg/ha)	Available Potassium (Kg/ha)
T_1	8.20^{a}	1.46^{c}	4.03^{c}	76.83^{a}	69.39^{c}	143.02^{e}
T_2	8.03^{ab}	1.76^{ab}	4.67^{b}	72.58^{c}	77.57^{a}	171.63^{c}
T_3	8.10^{ab}	1.23^{d}	4.28^{bc}	76.25^{ab}	69.59^{c}	163.22^{d}
T_4	8.03^{ab}	1.60^{bc}	4.08^{c}	76.40^{ab}	69.18^{c}	179.50^{b}
T_5	7.93^{b}	1.23^{d}	5.12^{a}	73.95^{bc}	74.07^{b}	196.97^{a}
T_6	7.90^{b}	1.86^{a}	5.41^{a}	72.66^{c}	69.61^{c}	202.03^{a}
T_7	7.56^{c}	1.56^{c}	4.48^{bc}	75.18^{b}	77.29^{a}	142.92^{e}
T_8	7.70^{c}	1.70^{b}	5.52^{a}	70.33^{d}	74.46^{b}	141.66^{e}
Mean	**7.93**	**1.55**	**4.70**	**74.27**	**72.64**	**167.62**
CD at 5%	**0 .19**	**0.11**	**0.40**	**1.54**	**2.32**	**7.47**
SEm±	**0.012**	**0.40**	**0.53**	**0.77**	**1.76**	**18.21**
CV	**1.37**	**4.09**	**4.91**	**1.19**	**1.83**	**2.55**

[T_1 (100% RDF-Recommended Dose of Fertilizers), T_2(50% RDF + PSB+ VAM), T_3(50% RDF + PSB+ Mustard cake), T_4 (50% RDF + PSB+ *Azotobacter*), T_5 (50% RDF + PSB+ VAM+ Mustard cake), T_6 (50% RDF + PSB+ VAM + *Azotobacter*), T_7 (50% RDF + PSB+ *Azotobacter*+ Mustard cake) and T_8 (50% RDF + PSB+ *Azotobacter*+ VAM+ Mustard cake)]

Table 2. Nutrient status of potato leaves after biofertilizers application to potato plants

Treatments	Nitrogen (g/Kg of DM)	Phosphorous (g/Kg of DM)	Potassium (g/Kg of DM)	Chl-A (mg/ml)	Chl-B (mg/ml)	Total chlorophyll (mg/ml)
T_1	41.88	3.993	80.533	2.842^{e}	5.108^{ef}	$7. 951^{f}$
T_2	44.32	4.616	78.863	8.344^{a}	7.229^{d}	15.57^{c}
T_3	42.99	3.650	78.876	4.885^{c}	5.816^{e}	10.70^{e}
T_4	45.42	3.596	80.536	3.567^{d}	4.454^{f}	8.022^{f}
T_5	42.30	3.833	81.316	8.927^{a}	2.231^{g}	11.15^{e}
T_6	44.78	3.876	81.433	2.561^{e}	15.36^{a}	17.92^{b}
T_7	42.79	4.620	79.913	1.762^{f}	11.13^{c}	12.89^{d}
T_8	48.77	5.833	86.626	7.064^{b}	13.88^{b}	20.951^{a}
Mean	**44.16**	**4.252**	**81.012**	**4.994**	**8.152**	**13.14**
CD at 5%	**NS**	**NS**	**NS**	**0.588**	**1.074**	**1.122**
SEm±	**8.50**	**0.786**	**27.477**	**0. 113**	**0.376**	**0.411**
CV	**6.60**	**20.85**	**6.47**	**6.73**	**7.53**	**4.88**

[T_1 (100% RDF-Recommended Dose of Fertilizers), T_2(50% RDF + PSB+ VAM), T_3(50% RDF + PSB+ Mustard cake), T_4 (50% RDF + PSB+ *Azotobacter*), T_5 (50% RDF + PSB+ VAM+ Mustard cake), T_6 (50% RDF + PSB+ VAM + *Azotobacter*), T_7 (50% RDF + PSB+ *Azotobacter*+ Mustard cake) and T_8 (50% RDF + PSB+ *Azotobacter*+ VAM+ Mustard cake)]

3.1.2. Electrical conductivity (EC)

A significant difference in EC of soil of potato field (Table 1) was reported between treatments, however no definite pattern can be extracted as per the observation. The highest value of EC (1.86 dS/m) was reported in T_6 (50% RDF + PSB+ VAM + *Azotobacter*) followed by 176 dS/m in T_2 (50% RDF + PSB+ VAM) and 1.70 dS/m in T_8 (50% RDF + PSB+ *Azotobacter*+ VAM+ Mustard cake). Most of the treatments containing biofertilizers has been reported with high values of electrical conductivity while T_3 (50% RDF + PSB+ Mustard cake) and T_5 (50% RDF + PSB+ VAM+ Mustard cake) reported EC of 1.23 dS/m and was lower than T_1 (1.46). The high value of EC in soil under biofertilizers application in combination with 50% NPK from RDF might be due to high moisture percentage and greater mobility of minerals in the soil solution which can be supported by the facts that biofertilizers creates acidic medium for better dissociation of mineral ions from soil mineral complex so can improve EC value. However, Zargar *et al.* (2008) had not reported any significant change in electrical conductivity in strawberry grown soil supplied with treatments containing *Azotobacter* and PSB.

3.1.3. Soil organic matter

Soil organic matter (Table 1) was significantly improved due to application of VAM, PSB, *Azotobacter* or mustard cake in combination with 50% NPK from RDF and highest organic matter (5.52%) was reported in T_8 (50% RDF + PSB+ *Azotobacter*+ VAM+ Mustard cake) followed by T_6 (5.40%) and T_8 (5.12%) however it was lowest (4.03%) in T_1 (100% N, P, K through RDF). High value of organic matter in soil supplied with biofertilizers might be due to the organic matter present in these sources of nutrients. This finding can be confirmed by the findings of Jatav *et al.* (2013) who had reported highest organic carbon due to integrated application of 50% P, K from inorganic fertilizers and 50% from FYM. Singh *et al.* (2016) had also reported improvement in soil organic matter due to combined application of vermicompost and inorganic fertilizers.

3.1.4. Total Nitrogen

Total nitrogen in soil (Table 1) was reported to vary significantly between treatments and was reported to be highest in T_8 (76.83 Kg/ha) followed by T_6 (76.40 Kg/ha), T_2 (76.25 Kg/ha) and T_7 (75.18 Kg/ha) in comparison to control (T_1) which is recorded with lowest (70.33 Kg/ha) Nitrogen content. This might be due to slow nutrient releasing tendency of these organic fertilizers which could be responsible for higher residual value of nitrogen in soil. The improvement in soil Nitrogen level can be justified by findings of Zargar *et al.* (2008) who had proposed highest available Nitrogen when 225 Kg N/ha and 150 Kg P/ha was applied in strawberry in combination with *Azotobacter.*

3.1.5. Available Phosphorus and Potassium

Data presented in Table 1 confirms that significantly highest available phosphorous (77.57 Kg/ha) was reported in T_2 (50% RDF + PSB+ VAM) followed by 77.29 Kg/ha in T_7 (50% RDF + PSB+ *Azotobacter*+ Mustard cake), 74.46 Kg/ha in T_8 (50% RDF + PSB+ *Azotobacter*+ VAM+ Mustard cake) and 74.07 Kg/ha in T_5 (50% RDF + PSB+ VAM+ Mustard cake) in comparison to 69.39 Kg/ha in T_1 (100% RDF).However, highest value of available K (202.03 Kg/ha) was reported in T_6 (50% RDF + PSB+ VAM + *Azotobacter*) followed by 196.97 Kg/ha in T_5 in comparison to 143.02 Kg/ha in T_1 (100% NPK through RDF). Thus, most of the treatments containing biofertilizers as source of nutrient has improved the value of available K which might be due to availability of P by phosphobacteria as compared by Bhattacharya *et al.* (2000). Similarly, application of PSB in combination with organic doses of fertilizers has been reported to improve P and K significantly as confirmed by Zargar *et al*. (2008).

3.2. Nutrients status of potato leaves

The observations (Table 2) clearly showed the non-significant influence of biofertilizers application on these attributes. However, highest P and K (48.77, 5.833 and 86.626) was reported in T_8 (50% RDF + PSB+ *Azotobacter*+ VAM+ Mustard cake) followed by 45.42 nitrogen in T_4 (50% RDF + PSB+ *Azotobacter*), 4.620 phosphorous in T_7 (50% RDF + PSB+ *Azotobacter*+ Mustard cake) and 81.433 potassium in T_6 (50% RDF + PSB+ VAM + *Azotobacter).* The higher value of nutrient in potato leaves might be due to better uptake of these nutrients however slightly lower level of N, P and K in comparison to higher expectation might be associated with efficient assimilation of absorbed nutrient by plant tissue for improved synthesis of starch and other bio molecules and high yield. The positive influence of biofertilizers (vermicompost) has also been reported by Singh *et al.* (2016) in Indian gooseberry and guava leaves and Dyson and Watson (1971) in potato leaves.

3.3. Chlorophyll content of potato leaves

Chlorophyll content of potato leaves was measured after 60 day of complete emergence (Table 2) which confirms that chlorophyll-A, chlorophyll-B and total chlorophyll significantly differed between treatments. Significantly highest (8.927 mg/ml) of chlorophyll- A was obtained in T_5 (50% RDF + PSB+ VAM+ Mustard cake) followed by 8.344 mg/ml in T_2 (50% RDF + PSB+ VAM) and 7.064 mg/ml in T_8 (50% RDF + PSB+ *Azotobacter*+ VAM+ Mustard cake) whereas chlorophyll-B content was reported to be highest (15.36 mg/ml) in T_6 (50% RDF + PSB+ VAM + *Azotobacter*) followed by 13.88 mg/ml in T_8 and11.13 mg/ml in T_7 (50% RDF + PSB+ *Azotobacter*+ Mustard cake). Total chlorophyll content was significantly highest (20.951 mg/ml) in T_8 followed by T_6 (17.92 mg/ml), T_2 (15.57 mg/ml) and T_7 (12.89) however, lowest (7.951 mg/ml) chlorophyll content was recorded in T_1. Thus, it is evident from the table and graph that there was significant effect of application of biofertilizers like PSB, VAM and *Azotobacter* on chlorophyll content of potato leaves. The high level of chlorophyll- A, chlorophyll- B and total chlorophyll content of potato leaves were might be due to greater uptake and utilization of nitrogen and improved availability of micro nutrients specially Mg which is an important constituent of chlorophyll unit. Although a number of works had been carried out on chlorophyll content affected by micronutrients but influence of biofertilizers over chlorophyll content is innovative approach of this investigation.

3.4. Tuber quality analysis

3.4.1. Nitrate content of Potato Tubers

The nitrate content of potato tubers grown under different treatments (Figure 1) confirmed that highest Nitrate (120 ppm) was reported in T_1 (control) followed by 103 ppm in T_3 (50% RDF + PSB+ Mustard cake) and 87 ppm in T_4 (50% RDF + PSB+ *Azotobacter*) which can be hazardous to the health. A significant reduction in nitrate level of potato tubers was reported due to application of combination of biofertilizers under various treatments. The lowest (36 ppm) value of nitrate was reported in T_8 (50% RDF + PSB+ *Azotobacter*+ VAM+ Mustard cake) followed by 37 ppm in T_6 (50% RDF + PSB+ VAM + Azotobacter) and 41 ppm in T_5 (50% RDF + PSB+ VAM+ Mustard cake). T_3 (50% RDF + PSB+ Mustard cake) and T_7 (50% RDF + PSB+ *Azotobacter*+ Mustard cake) had also reported safe nitrate level of 62 ppm and 65 ppm respectively. Reduction of nitrate level in potato tuber due to application of biofertilizers in combination with 50% dose of RDF might be due to lower dose of nitrogenous fertilizers applied in all treatments except T_1, this result may also be associated with slow nitrogen releasing habit of *Azotobacter*, VAM or Mustard cakes so rate of assimilation of nitrogen and its absorption by plant were in equilibrium to avoid accumulation of nitrate in tubers. The present findings and reasons are in conformity with the findings of Carter and Bosma (1974). However, high nitrate in T_1 can be justified by Byrne *et*

al. (2001); Tittonell *et al.* (2003) and Parente *et al* (2006) who had confirmed that application of excess of nitrogenous fertilizers disrupted the equilibrium between absorption, transportation and utilization of nitrogen to cause accumulation of nitrates in leaves of lettuce.

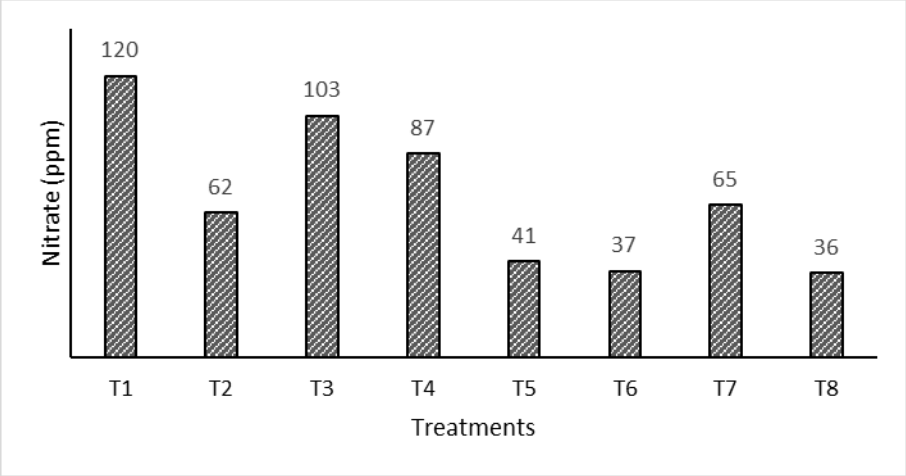

Figure 1. Nitrate (ppm) content of potato tubers under various treatments

[T_1 (100% RDF-Recommended Dose of Fertilizers), T_2(50% RDF + PSB+ VAM), T_3(50% RDF + PSB+ Mustard cake), T_4 (50% RDF + PSB+ *Azotobacter*), T_5 (50% RDF + PSB+ VAM+ Mustard cake), T_6 (50% RDF + PSB+ VAM + *Azotobacter*), T_7 (50% RDF + PSB+ *Azotobacter*+ Mustard cake) and T_8 (50% RDF + PSB+ *Azotobacter*+ VAM+ Mustard cake)]

3.4.2. Tuber Nutrient Content

The mineral content of potato tuber has been presented through Figure 2 to Figure 5. These figures confirmed that application of biofertilizers in combination of 50% nutrient through RDF had significantly improved the K, Ca, Zn, and Fe level in skin and flesh of potato tubers. However, the concentration of nutrients was reported to be higher in skins in comparison to flesh.

The highest K level (39.1mg/g) in skin and (23.6 mg/g) in flesh of T_8 (50% RDF + PSB+ *Azotobacter*+ VAM+ Mustard cake) and T_5 (50% RDF + PSB+ VAM+ Mustard cake) respectively followed by 38.6 mg/g in skin and 23.6 mg/g in flesh of T_6 (50% RDF + PSB+ VAM + *Azotobacter*). Overall T_8 (50% RDF + PSB+ *Azotobacter*+ VAM+ Mustard cake) has been recorded with highest (31.25 mg /g) of average K content in whole tuber, however T_1 (control) was recorded lowest (31.4 and 20.23 mg/g) K in skin and flesh respectively. Maximum Calcium (2.39 and 0.32 mg/g respectively) was determined in skin and flesh of T_8 (50% RDF + PSB+ *Azotobacter*+ VAM+ Mustard cake) followed by 2.36 mg/g Ca in skin and 0.62 mg/g Ca in flesh of T_6 (50% RDF + PSB+ VAM + *Azotobacter*), however lowest (1.9 mg/g and 0.24 mg/g) was reported in skin and flesh of T_1 (100 nutrients through RDF), respectively. Similarly, T_5 (50% RDF + PSB+ VAM+ Mustard cake), T_8 (50% RDF + PSB+ *Azotobacter*+ VAM+ Mustard cake) and T_6 (50% RDF + PSB+ VAM + *Azotobacter*) have been recognised with highest (9.7,9.66 and 9.65 µg/g; respectively) level of Zn in flesh whereas highest (33.8 µg/g) Zn content in skin was determined in T_8 (50% RDF + PSB+ *Azotobacter*+ VAM+ Mustard cake) followed by 33.6 µg/g in T_6 (50% RDF + PSB+ VAM + *Azotobacter*) and 32.3 µg/g in T_5 (50% RDF + PSB+ VAM + Mustard cake). The lowest (30.3 µg and 8.9 µg) Zn content in skin and in flesh, respectively was reported in T_1 (Control). Likewise, highest (307.0 µg/g and 16.2 µg/g)

Fe content was reported in skin of T_5 (50% RDF + PSB+ VAM+ Mustard cake) and flesh of T_8 (50% RDF + PSB+ *Azotobacter*+ VAM+ Mustard cake), respectively while lowest was in T_1 (control) which contains only 14.6 µg/g of Fe in flesh and 306.6 µg/g in skin. Fe content of skin was not reported to be significant whereas Fe content of flesh was significantly higher in biofertilizers applied treatments in comparison to control (T_1). The highest level of K due to application of PSB in combination with *Azotobacter* or VAM might be due to efficient absorption and translocation of K from soil in presence of advantageous microbes associated with these biofertilizers. Similarity availability of micronutrients might have been improved due to release of these nutrients from complex soil micelle in presence of biofertilizers so uptake, had also been improved as reported by Parmar and Dufresne (2011). Yao *et al.* (2002) has also reported increased K level in plant tissue when potato tubers were inoculated with *G. etunicatum* (VAM fungi).

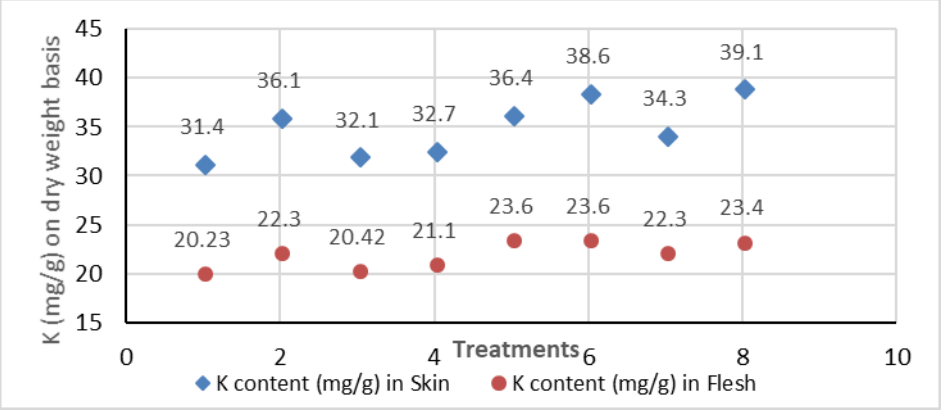

Figure-2: Potassium (mg/g) content of potato tubers under various treatments

[T_1 (100% RDF-Recommended Dose of Fertilizers), T_2(50% RDF + PSB+ VAM), T_3(50% RDF + PSB+ Mustard cake), T_4 (50% RDF + PSB+ *Azotobacter*), T_5 (50% RDF + PSB+ VAM+ Mustard cake), T_6 (50% RDF + PSB+ VAM + *Azotobacter*), T_7 (50% RDF + PSB+ *Azotobacter*+ Mustard cake) and T_8 (50% RDF + PSB+ *Azotobacter*+ VAM+ Mustard cake)]

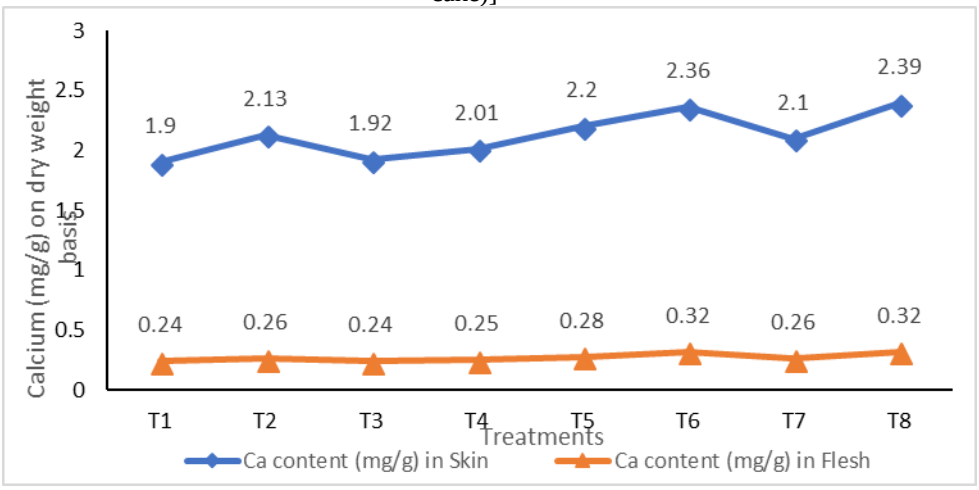

Figure-3: Calcium (mg/g) content of potato tubers under various treatments

[T_1 (100% RDF-Recommended Dose of Fertilizers), T_2(50% RDF + PSB+ VAM), T_3(50% RDF + PSB+ Mustard cake), T_4 (50% RDF + PSB+ *Azotobacter*), T_5 (50% RDF + PSB+ VAM+ Mustard cake), T_6 (50% RDF + PSB+ VAM + *Azotobacter*), T_7 (50% RDF + PSB+ *Azotobacter*+ Mustard cake) and T_8 (50% RDF + PSB+ *Azotobacter*+ VAM+ Mustard cake)]

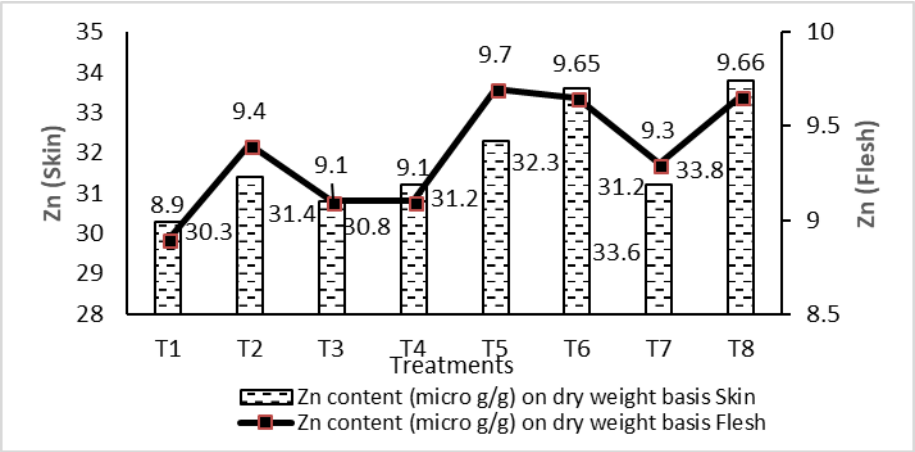

Figure-4: Zinc (µg/g) content of potato tubers under various treatments

[T_1 (100% RDF-Recommended Dose of Fertilizers), T_2(50% RDF + PSB+ VAM), T_3(50% RDF + PSB+ Mustard cake), T_4 (50% RDF + PSB+ *Azotobacter*), T_5 (50% RDF + PSB+ VAM+ Mustard cake), T_6 (50% RDF + PSB+ VAM + *Azotobacter*), T_7 (50% RDF + PSB+ *Azotobacter*+ Mustard cake) and T_8 (50% RDF + PSB+ *Azotobacter*+ VAM+ Mustard cake)]

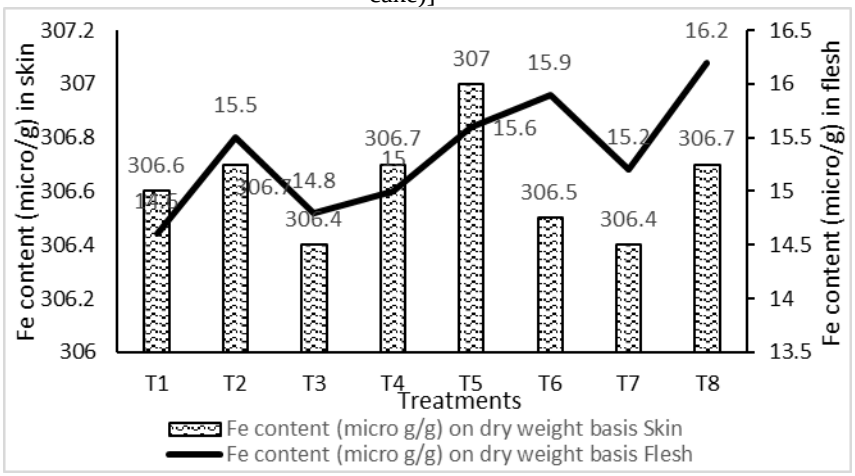

Figure-5: Iron (µg/g) content of potato tubers under various treatments

[T_1 (100% RDF-Recommended Dose of Fertilizers), T_2(50% RDF + PSB+ VAM), T_3(50% RDF + PSB+ Mustard cake), T_4 (50% RDF + PSB+ *Azotobacter*), T_5 (50% RDF + PSB+ VAM+ Mustard cake), T_6 (50% RDF + PSB+ VAM + *Azotobacter*), T_7 (50% RDF + PSB+ *Azotobacter*+ Mustard cake) and T_8 (50% RDF + PSB+ *Azotobacter*+ VAM+ Mustard cake)]

4. Conclusions

The treatments T_6 (50% RDF + PSB+ VAM + Azotobacter), T_7 (50% RDF + PSB+ Azotobacter+ Mustard cake) and T_8 (50% RDF + PSB+ Azotobacter+ VAM+ Mustard cake) were reported with low soil pH; high soil organic matter; high availability of nitrogen, phosphorus and potassium; lower nitrate content in tuber; and high mineral content in both pulp and skin. Thus, replacement of 50% of RDF with application of PSB in combination with VAM, Azotobacter or Mustard cake is most suitable INM practice for good soil health, lower nitrate toxicity and better tuber quality in potato grown under subtropics.

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FUNCTIONAL PROPERTIES OF FULL FAT DIKA KERNEL (*IRVINGIA WOMBOLU*) FLOUR

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ABSTRACT
The study investigated the functional properties of dika kernel (*Irvingia wombolu*), an African food hydrocolloids used to flavour and thicken soups. The kernel flour was analysed using standard methods. The result showed that the flour has potential in functional food systems. The pH of the dika kernel (5.34) may not encourage the growth of spoilage organisms. The kernel would make a good thickening and gelling agent in related food systems. The flour is characterised by its high water absorption rate (343 and 492%) during reconstitution. It might serve as a good forming agent and useful for stabilisation of emulsion in soups. The foaming and emulsion properties of the samples studied were pH dependent and were influenced by salt (NaCl) concentration. The kernel also contains significant amount of digestible protein (about 90%) for human nutrition. The study suggested that dika kernel (*Irvingia wombolu*) be explored in industrial applications regarding functional foods.

1. Introduction

Irvingia wombolu is from the family *Irvingiaceae*, known as bitter bush mango/dika kernel. It is a variety of an African indigenous hydrocolloids, mainly used for culinary purpose in the thickening of soups and stews (Ndjouenkeu *et al*., 1996). Dika kernel properties are like the functional properties of acacia gum, as it has the capacity to solubilise in water and produce high viscosity. The behaviour of proteins in foods are affected by their physicochemical properties. The functionality of protein depends on the size and structure of proteins and their interactions with other food components such as carbohydrates and fats.

The supplies of food proteins can be increased be exploiting protein from wild plants for use as functional food ingredients. There is little information on the functional properties of *I. wombolu*. This information is essential for determining potential uses in food formulations and will add value to this important African hydrocolloid. The study therefore, aimed to determine the functional properties of *Irvingia wombolu* kernel flour, to increase its application industrially.

2. Materials and methods

2.1. Materials

Irvingia wombolu fruits were obtained from Agbeonu-Obey farm, near Ile-Ife, Nigeria. The variety was identified by its mesocarp, tree and season of harvest, and was confirmed at the Herbarium, Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria. The kernel was removed from the fruit, skinned, cleaned and sorted to remove unwanted materials. The dika kernel was sun dried to about 8 ± 2% moisture content, and milled using a laboratory mill (3303, Sweden). The ground kernel was passed

through the first three mm sieves for uniformity using the Endecotts Test Sieve Shaker (SN 9229, Endecott Ltd, England) according to ASAE Standard S319.3 (ASAE, 2003). The samples retained on the 1 mm aperture sieve was packed in plastic bags and placed in a desiccator for further analysis. All reagents were of analytical grade, obtained from the Food Chemistry Laboratory, Department of Food Science and Technology, Obafemi Awolowo University, Ile-Ife, Nigeria.

2.2. Methods

Functional properties of the flour evaluated were pH, bulk density, water and oil absorption capacity, least gelation concentration, foaming capacity and stability, emulsifying activity index and emulsion stability.

pH was measured using the pH meter (Scholar 425, Corning Electrode 14831, New York) already standardised at 25 with buffer solution of pH 4 and 7. Bulk density was determined according to the method of Okezie and Bello (1988). The sample was centrifuged (Hospibrand 0502-1, U.S.A) at 3000 rpm for 25 min, the water absorption capacity was determined between ambient temperature and

90 according to the method of AACC (2000); the oil (Devon King's vegetable oil, Malaysia) absorption capacity was determined using the method of Beuchat (1977) with slight modification.

Methods of Sathe and Salunkhe (1981) and Sathe *et al*. (1982) were used to determine least gelation concentration (LGC). The sample solution was homogenised for 2 min using a blender (Marshal Hex Okapi, China) set at high speed, the foam capacity and stability as influenced by pH and salt concentration was determined by a modification of the method described by Chavan *et al*. (2001) and Ogunwolu *et al.* (2009). The effect of pH and salt concentration on emulsifying activity index (EAI) was determined by the method of Gbadamosi et al. (2012) with slight modifications using spectrophotometer (Spectrumlab 752S, UV-VIS Spectrophotometer, China).

In vitro protein digestibility (IVPD) of the flour was measured according to the modified method of Chavan *et al*. (2001) using a controlled environment incubator shaker (New Brunswick Scientific 3.430.926, U.S.A).

3. Results and Discussions

3.1. Functional Properties of the Dika Kernel

The particle size analysis showed that dika kernel powder had difficulty passing through the sieve aperture. This difficulty may be due to the presence of lipids interfering in the particle size reduction during the kernel grinding. This is similar to the report that particle adhesion during grinding of oilseeds poses difficulty in obtaining uniform particle sized flour (Joshi *et al*., 2015). The results of the functional properties of dika kernel are presented in Table 1.

Table 1. Functional Properties of Dika Kernel

Property	**Dika kernel**
In-Vitro Protein Digestibility (%)	89.90 ± 1.17
pH	5.34 ± 0.01
Bulk Density (g/cm^3)	0.57 ± 0.02
Least Gelation Concentration (%, w/v)	
1	-
5	±
10	+
15	+
20	+

Water Absorption Capacity (%)	
RT	343 ± 0.29
45	401 ± 0.42
60	492 ± 0.63
75	449 ± 0.57
90	440 ± 0.52
Oil Absorption Capacity (%)	49 ± 0.19

Values reported are means ± standard deviation of triplicate determinations; RT: Room Temperature

The pH of the dika kernel was 5.34. The value was slightly acidic, indicating the presence of some organic acids. This pH level may, therefore, inhibit the growth of some spoilage organisms during storage. Information on the pH of flour is important since some functional properties such as solubility, emulsifying and foaming properties are affected by pH (Gbadamosi *et al*., 2012). The bulk density of dika kernel was 0.57g/cm^3. The presence of lipid in the dika kernel may be responsible for this value, this is because the particle may fold nearer to the triglycerides, acting as adhesives in the accumulation of the carbohydrate and protein molecules, permitting higher bulk density (Joshi *et al*., 2015). Bulk density measures heaviness of a flour sample (Oladele and Aina, 2007). It indicates the behaviour of a food product in dry mixes with variation in finess of the particles. Particle size and packing density majorly contribute to the determination of bulk density of flours. Bulk density is an indication of the porosity of a product which influences package design and could be used in determining the type of packaging material required. The least gelation concentration (w/v) showed that the dika kernel exhibited partial gelation at 5% and full gelation at 10% and above. The results showed that dika kernel would make a good thickening agent and will be useful in food systems that require thickening and gelling. The results observed are different from those reported by Ogungbenle (2014) for raw and defatted *Igabonensis* (12 and 14 % w/v respectively) but compared well with the value (10%) reported by Abulude *et al*. (2008). The values obtained in the study are within the range reported for production of curd or additive to other materials for forming gel in food products. The water absorption capacity (WAC) of dika kernel varied between 343 and 492%. The values are higher than those reported for raw *I. gabonenesis* (241%) and defatted *I. gabonensis* (249%) (Ogungbenle, 2014). This may be due to varietal differences. The values are also higher than those reported for *I. gabonensis* (150%), *okra* (241.6 – 269.5%), cowpea (256%), and defatted *Kariya* (124.37%) (Abulude *et al*., 2008; Adelakun et al., 2010; Odedeji and Oyeleke, 2011; and Adebayo etal., 2013). The hydrophilic nature of the dika kernel (*I. wombolu*) suggests its usefulness in the production of soups and gravies. Lawal and Adebowale (2004) explained that the major components that enhance water absorption capacity of flour are protein and carbohydrates since these constituents contain hydrophilic parts such as polar or charged side chains. High water absorption is desirable in the dika kernel because of its high affinity for water during reconstitution. Increase in temperature to 60 caused an increase in water absorption of the dika kernel. pigeon pea (5-10%) by Onweluzo and Nwabugu (2009). This information suggests the potential usefulness of dika kernel in the production of curd or additive to other materials for forming gel in food products. This is expected because as the temperature is increased, the starch components of the dika kernel begin to imbibe water, resulting in the leaching out of amylose. This results in swelling and higher WAC. Further increase in the temperature to 90 reduced water absorption of the flour. Temperature above 60can cause starch gelatinization and protein denaturation (Eckhoff and Tso, 1991), thereby resulting in lower WAC. Water absorption is an important processing parameter in the processing of food, as it affects

the viscosity, bulk and consistency of food products (Niba *et al*., 2001). The result of the OAC of dika kernel was 49%. Low hydrophobic protein showing superior binding of lipid could be responsible for the lower oil absorption capacity (Adejuyitan *et al*., 2009) of dika kernel. Binding of the oil depends on the surface availability of hydrophobic amino acids. Increase in the availability of these amino acids through the exposing of the non-polar residues from the interior protein molecules also contribute to high OAC. The mechanism of oil absorption is attributed mainly to the physical entrapment of oil and the binding of fat to the polar chain of protein (Wang and Kinsella, 1976). In food formulation, OAC plays a vital role in increasing the mouth feel of foods and acts as a flavour retainer (Kinsella, 1976a). OAC also improves the palatability particularly in bakery or meat products where fat absorptions are desired (Aremu *et al*., 2007).

3.2. Effects of pH and salt on foaming capacity and stability of dika kernel

Effects of pH and NaCl concentration on foaming capacity (FC) and foaming stability (FS) of dika kernel are presented in Figure 1 (a-b). The results showed that the FC of dika kernel ranged between 1.54 and 19.06%, and the FS of dika kernel ranged from 2.08 to 37.04%. During whipping, protein is denatured and aggregated due to the increase in the surface area of the water/air interphase. The amount of solubilized proteins after whipping increased, thereby, resulting in an increased FC of the dika kernel. The dika kernel in its natural state had a foaming capacity of 11.04 ± 0.66%. This favourably compares with the FC of defatted *I. gabonensis* seed (11.0%) reported by Ogungbenle (2014). The FC and FS of dika kernel obtained in this study are within the range 5 - 30.5% and 5 - 65% respectively, reported for *okra* by Adelakun *et al*. (2010).

The FC and FS of the flour were lowest at pH 6 presumably the isoelectric point. This could be due to the minimum solubility of the protein near the isoelectric point, which is attributed to minimum repulsion among the constituent amino acids. The balance in positive and negative charges decreased the electrostatic repulsion, and this, therefore, may contribute to reduced foaming properties at isoelectric pH (Kain *et al*., 2009). Away from the isoelectric point, foaming properties increased at both the acidic and alkaline region. This is due to the increase in both the net charge of the peptides as pH moves away from the isoelectric point (Sorgentini and Wanger, 2002). Similar observations have been reported by Sathe *et al*. (1982a) for winged bean flour.

The FC and FS of the dika kernel were found to increase as salt concentration increased from 0.0 M to 0.5 M. These increases could be attributed to salting-in-effect of protein where the salt have the ability to enhance the surface charges on proteins and hence cause an increased FC and FS (Hui, 2006). However, at the acid region, a decrease in FC and FS was observed as the salt concentration increased from 0.5 M to 1.0 M. This might be as a result of the salting-out effect of NaCl. Salting-out occurs at high salt concentrations when salts compete with the protein for water (Hui, 2006). The addition of salt at high concentration causes negatively charged chloride ions to interact with the positively charged proteins. This thereby decreases electrostatic repulsions and enhances hydrophobic interactions (Ahmed *et al*., 2012). The increase in hydrophobic interactions would result in a higher tendency for the protein to form insoluble aggregates, thus decreasing solubility (Aluko and Yada, 1993) and hence, decrease in FC and FS.

The results agree with those in earlier studies where it was stated that FC and FS are improved with the addition of salts, phosphoric acid, citric acid and metallic ions (Stadelman and Cotterill, 1994; Hatta *et al*, 1997). In foods, sodium chloride is commonly used for this purpose. Improved FC and FS in the presence of NaCl will enhance dika kernel functionality and its application in food where foaming is of vital importance.

This result also showed that FC and FS of the dika kernel were pH dependent, and performed better in the alkaline region. This agrees with the report of Khalid and Elhardallou (2015) that

higher values of the FC and FS of cowpea flour were observed at alkaline region compared to acidic side. FC and FS are reported to depend on pH, as it was observed in winged beans, lupin, sunflower and cowpea seed protein (Lin *et al.*, 1974; Sathe *et al.*, 1982; Aluko and Yada, 1995). Foam stability is important in food systems because its application depends on the ability to maintain whipping process as long as possible (Boye *et al.*, 2010). Foaming capacity enhances the functionality of seeds in the production of cakes, whippings, and toppings (Kinsella, 1976b). Since the dika kernels have a good foaming capacity and stability, they might serve as good foaming agents in food requiring foaming. Uzomah and Ahiligwo (1999) confirmed this by exploring the possibility of *I. gabonensis* gums in the formulation of an ice- cream mix.

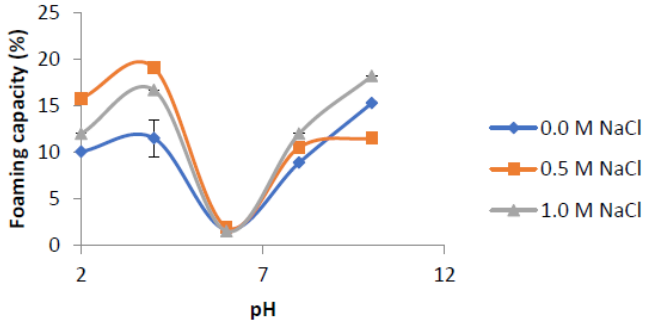

Figure 1a. Effect of pH on foaming capacity of dika kernel at 0.0, 0.5 and 1 M NaCl concentrations

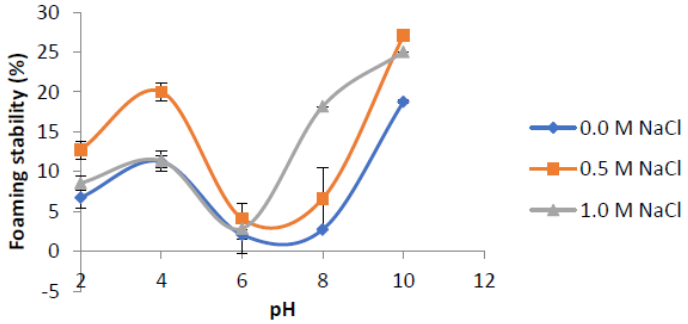

Figure 1b. Effect of pH on foaming stability of dika kernel at 0.0, 0.5 and 1 M NaCl concentrations

3.3. Effects of pH and salt on emulsion activity and stability indices of dika kernel

The effects of pH and salt concentration on emulsion activity and stability indices of dika kernel are presented in Figures 2a-b. The Emulsifying Activity Index (EAI) of dika kernel ranged between 1.03 and 7.63 m2/g, while the Emulsion Stability Index (ESI) ranged between 46.45 and 249.77%. Protein concentration is an important factor that strongly affects emulsifying ability, proteins with a large number of hydrophobic groups on their surface tends to be greater emulsifiers (Hui, 2006). This means that high content of hydrophobic amino acids in the protein improves surface hydrophobicity. The exposed hydrophobic groups improved interaction between the protein and the lipids of the sample, thereby resulting into higher EAI and ESI of the ready-to-cook soup mix.

EAI and ESI of the dika kernel increased with the addition of NaCl concentrations. Addition of NaCl from 0.0 to 1.0 M concentration increased the EAI of the flour. Increase in EAI with increase in the salt concentration up to 0.5 M enhanced the formation of charged layers around the protein globules, resulting in mutual repulsion among them. However, beyond 0.5 M NaCl concentration ESI decreased. This effect

may be due to an increase in the net charge of the protein by the addition of NaCl which weakens hydrophobic interaction and increases protein solubility and flexibility. Therefore, allowing the protein to spread to air- water interface more quickly, encapsulating lipid droplet and thus increasing emulsion formation (Lawal et al., 2004). The same trend was observed in the emulsion activity of soybeans flour reported by Berhanu and Amare (2013). A similar observation of Osman et al. (2005) reported that the addition of NaCl decreased the ESI of untreated chickpea flour.

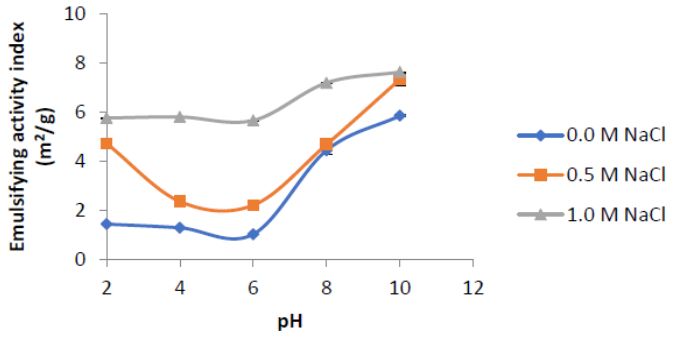

Figure 2a. Effect of pH on EAI of dika kernel at 0.0, 0.5 and 1 M NaCl concentrations

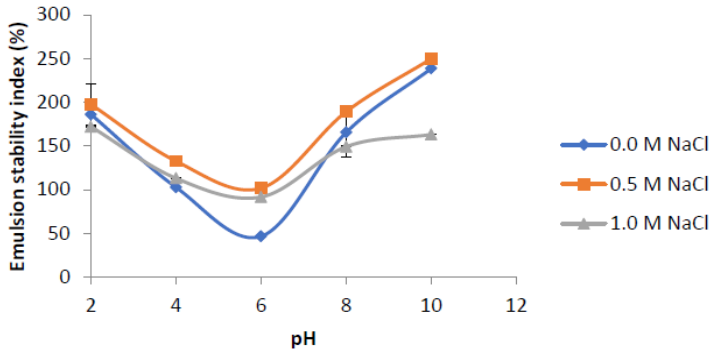

Figure 2b. Effect of pH on ESI of dika kernel mix at 0.0, 0.5 and 1 M NaCl concentrations

Increased EAI with an increase in NaCl concentration showed that the salt concentrations may selectively affect the formation of emulsion (Hui, 2006) of *I. wombolu* kernel. The result of ESI obtained in this study at 0.0 M and 0.5 M NaCl concentration of the sample, reflect the good emulsifying property of the flour where its ability to maintain the emulsion on subsequent processing steps such as cooking and canning depends on (Ahmed *et al*., 2011). The trend observed in this study indicated that EAI and ESI of the flour were pH dependent. The sample exhibited minimum emulsifying properties at pH 6. This is because proteins near isoelectric point are poor emulsifiers due to their poor solubility (Hui, 2006). Away from the isoelectric point, emulsifying properties of the flour improved.

The EAI and ESI values obtained in this study are higher than those reported (44 and 30.5% respectively) for raw okra seed flour (Adelakun *et al*., 2010). Emulsifying properties are important functional properties of flour. They help in fat/water phase stability in many baked food products. Aremu *et al*. (2008) reported that emulsion capacity/stability and foaming capacity/stability of Bambara flour were affected by salt and the effect depends on the type of salt and their concentrations. This confirms why salt may be used to improve the functional property of dika kernel. Emulsion properties play a

significant role in many food systems where the proteins have the ability to bind fat such as meat product, dough, and so on (Sathe and Salunkhe, 1981; Adeleke and Odedeji, 2010). The emulsion capacity of a protein is a function of the concentration and the type of proteins having high surface hydrophobicity and adsorption at the oil and water interface (Sikorski, 2002). Protein can emulsify and stabilise the emulsion by decreasing the surface tension of the oil droplets and providing electrostatic repulsion of the surface of the oil droplet (Regena *et al*., 2013). This result suggests that dika kernel might be useful in food formulation as an additive for the stabilisation of emulsion in soups.

3.4. *In vitro* protein digestibility

In vitro protein digestibility (IVPD) of the dika kernel was 89.90%. The IVPD of dika kernel was higher than that reported for *conophor* flour (52.28%) by Gbadamosi et al. (2012) and *Kariya* flour (72.36%) by Adebayo et al. (2013). This higher IVPD value of dika kernel may suggests the presence of pepsin-pancreatin resistant anti-nutrients, making it easily digestible. The result showed that dika kernel contains a significantly high amount of digestible proteins for human nutrition.

4. Conclusions

Functional properties of *Irvingia wombolu* showed high affinity for water during reconstitution, good foaming capacity and stability. The foaming and emulsion properties studied reduced with an increase in pH until the isoelectric pH was reached, then further increased with increase in pH. The addition of salt (NaCl) at different concentrations improved the foaming and emulsion properties studied.

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EFFECT OF APPLE POMACE POWDER ON RHEOLOGICAL PROPERTIES OF DOUGH AND SANGAK BREAD TEXTURE

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ABSTRACT
Apple pomace (AP), as waste of food processing plants and abundant and cheap source of fiber, has special advantages such as lack of phytic acid; hence, it has the potential to be used in bread making. This article aims to study the effect of adding four levels of Apple Pomace Powder (APP) (1 %, 3 %, 5 %, and 7 % w/w of flour) on rheological properties of dough and Sangak bread texture during storage. The results showed that adding APP led to reduced hardness. The results of image analysis also showed that crust color of bread with APP was darker compared to the control sample. However, sensory evaluation shows that adding APP up to 3% is completely acceptable for consumers in comparison with control sample. It also left positive effects on aroma and texture of Sangak bread. The whole study indicates that AP can be used as additives in Sangak bread making to correct the rheological and organoleptic properties and fortify the diet.

1. Introduction

Bread is one of the most-widely used products worldwide. On the other hand, staling quickly reduces the quality of bread. Staling is a complex process which has widely been studied (Zobel and Kulp, 1996). Multiple methods have been introduced to reduce staling such as using gums and fibrous material in the formulation of bread (Karim et al., 2000; Majzoobi et al., 2011). Fiber is a general term which refers to a wide range of non-starchy carbohydrates and lignin (Nawirska and Kwasniewska, 2005). Waste of fruit and vegetable processing plants is one of abundant and cheap sources of fibers. Tomato, beet, carrot, apple, fruit peel pomace are some of the most important sources of rich fibers (Nawirska and Kwasniewska, 2005; Shah and Masoodi, 1994). In addition to nutritional value increase, dietary fibers change the bread rheological properties. They are also effective in bread quality and sensory characteristics (Gomez et al., 2003).These compounds are divided into soluble and insoluble in water (Mckee and Latner, 2000). Insoluble dietary fibers (cellulose, lignin, and hemicellulose) shorten the stomach-intestinal transfer time and prevent

constipation. Daily use of fiber in food dietary reduces the colon cancer (Nawirska and Kwasniewska, 2005; Nawirska and Uklanska, 2008). In addition to high fiber and balanced ratio of soluble and insoluble fibers, apple has unique advantages compared to cereals bran and herb crust including lack of phytic acid (Larrauri, 1999; Mckee and Latner, 2000; Yam and Papadakis, 2004). APP was successfully used in floury products such as muffin, cookies, and cakes. The results show volume reduction, increased weight of loaf of bread, water absorption, brown crust, and firm texture (Joshi and K., 2001; Masoodi et al., 2002; Mckee and Latner, 2000).

Sangak is a type of Iranian bread, its flat triangular or rectangular in shape bread made to be about 70-80 cm long baked in a unique way. The name means "little stones" and the full name of the bread, "nan-e sangak" translates to "little stone bread." Sangak is made of very thin sheets of yeast dough which are placed on the bottom of an oven or a baking sheet covered with small river rocks. The oven is dome-shaped and heated with a natural gas burner and bread is baked for 3–5 min. The bread weight is about 400 g and due to high moisture content, stales faster than other Iranian breads. Traditional sangak has a slightly sour flavour and a distinctive spongy texture.

This article aims to evaluate the quality and delayed staling of Iranian traditional bread, Sangak, by adding APP considering the abundant source of waste of apple processing plants and high dietary fibers through rheological and organoleptic properties of product compared to the control sample.

2. Materials and methods

2.1. Materials

Wheat flour (Khusheh Flour, Shiraz, Iran) with an extraction rate of 93 percent, Apple pomace (Keshavarz Orange juice, Fars, Iran) and salt (Golha, Tehran, Iran) were purchased from the market. All chemicals used to chemical analysis were of analytical grade and they were purchased from Merck Co.

2.2. Apple Pomace Preparation

Apple pomace was dried in a cabinet dryer at 58 °C. After separating the seeds and stems, apple pomace was grounded by hammer mill (Marconi, MA 680; Piracicaba, Brazil). After sifting with 50 mesh, it was employed to bake bread (Masoodi et al., 1998).

2.3. Baking

Three kg wheat flour, 45 grams of salt, and essential amount of water were employed to prepare the dough.20% sourdough was added to the prepared dough. Apple pomace (1%, 3%, 5%, and 7%) was added while mixing. Finally, it was kept for 2 hours at 30 °C for fermentation. Such dough was traditionally baked in bakeries at 350-500 °C for 2-4 minutes (Qarooni, 1996).

2.4. Chemical Analysis

The composition analysis in wheat flour, which consisted of moisture, protein, ash, fat and fiber performed by AOAC 925/10 (2000), AOAC 920/87 (2000),AOAC 923/03 (2000), AOAC 922/06 (2000) and AOAC 991/43 (2000), respectively. Moisture, protein, ash, fat and fiber contents of apple pomace were determined according to AOAC (2000) official methods.

2.5. Dough rheological properties

Texture analyzer CT3 model (Brookfield Engineering. Middle Boro, USA) and Grausgruber et al. (2003) model were employed to determine the rheological properties (Grausgruber et al., 2003). TA-DSJ fixture and TA-3/1000 probe were used to clarify the properties. Probe was placed at speed of 0.5 mm/second over the dough extruding from the fixture. Then the highest force in the negative section of the chart and the distance where dough passes in backward mode of probe from fixture are determined as dough adhesive force and extensibility (Bourn, 1978). This was repeated three times and the mean score was reported for each sample.

2.6. Texture feature of fresh and kept [non-fresh] bread

To analyze the texture (Punch test and texture profile), texture analyzer, CT3 model (Brookfield Engineering. Middle Boro, USA) was employed.

2.6.1. Punch test

In punch test, probe TA /44 has a diameter of 4 mm and penetrates to the depth of 7 mm at a speed of 0.5 mm/second. The highest force recorded by texture analyzer was considered the hardness. This was repeated for three different sections of bread in each treatment.

2.6.2. Texture profile analyzer

In texture profile analysis test of probe TA/25/1000 (with diameter of 50 mm and speed of 0.5 mm/s), a pressure equal to 25 % was imposed on samples in two stages. Hardness and cohesiveness were then measured by texture analyzer.

2.7. Image Analysis

Crust color analysis was performed by Yum et al. (2004) method using a digital camera (Canon PC 1585, 14 Megapixel, China). The distance to samples and the angle were 30 cm and 90 degrees, respectively. Image analysis was performed by Image Pro Plus where L (lightness), a (green-red interval), and b (yellow-blue interval) were determined (Yam and Papadakis, 2004).

2.8. Sensory Evaluation

Aroma, texture, taste, color, appearance, and acceptability of breads with different levels of apple pomace were studied by 20 panelists. For sensory evaluation, Hedonic scoring system was used in which the highest score (5) was assigned to the best and lowest points (1) was assigned the worst example (Watts et al., 1989).

2.9. Information and statistics analysis

In this study, 5 treatments were performed in a completely randomized design with three replications. ANOVA and Duncan test were employed for data analysis using SPSS Ver. 21.

3. Results and Discussion

3.1. Chemical Analysis

Table 1 shows the chemical analysis of flour and APP, including moisture, ash, fat, protein, and fiber. The results show that APP fiber and ash is far more than wheat flour.

Table 1. Wheat flour and APP chemical analysis

Chemical composition (% dwb)	Moisture	Fiber	Protein	Fat	Ash
Wheat flour	12.33	0. 73	10.81	1.22	0. 83
APP	10.5	14.5	1.2	0.6	2.5

dwb Dry weight basis, APP Apple Pomace Powder

3.2. Rheological properties of dough

Figure 1 showed that APP increase leads to significant increase and decrease in adhesive force and extensibility of dough, respectively ($P<0.05$). Concerning the dough extensibility, no significant difference is found in samples with different amounts of APP. Adhesive force is significantly higher than other treatments in 7 % treatment ($P<0.05$). In other words, the highest adhesive force and the lowest extensibility belong to 7 % treatment. The highest extensibility and the lowest adhesive force belong to control sample.

The results of rheological properties of dough are associated with gluten network destruction or reaction between the polysaccharide and protein, and accordingly, reduced extensibility of dough (Chen et al., 1988; Wang et al., 2002). These results are similar to those of Gomez et al. (2003) who studied the effect of fiber on rheological

properties of dough and bread (Gomez et al., 2003).

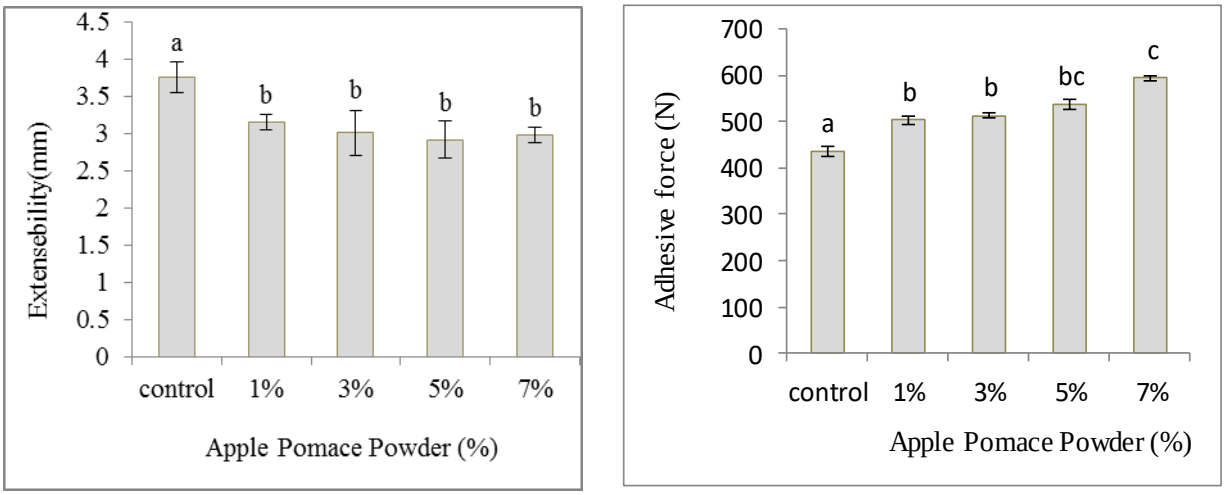

Figure 1. Adhesive force and extensibility of dough at different levels of APP

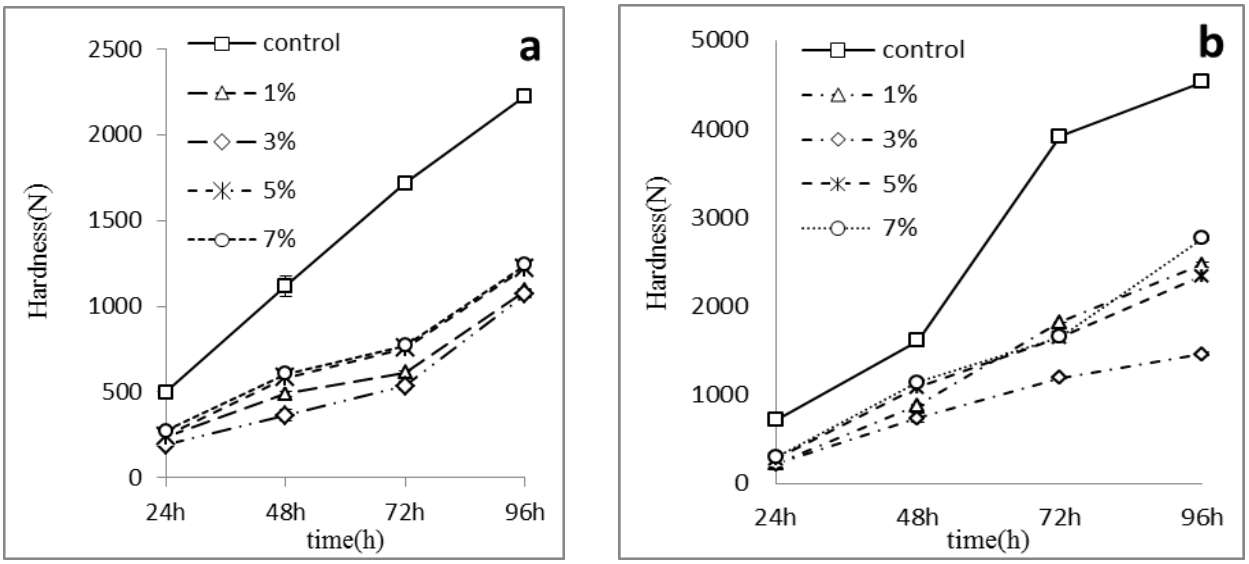

Figure 2. Comparison of APP and time on hardness resulting from a) penetration test, b) TPA test

3.3. Bread texture properties

TPA test, which consists of two compression cycles, is used to determine three parameters such as hardness. On the other hand, penetration test is a solution to simulate the bread cutting force in chewing (200). A high correlation coefficient was observed for hardness in Punch Test and Texture Profile Analyzer. There was, for example, a 99.9 % significant correlation coefficient for harness (24 hour keeping duration) in both tests (r=0.991).

Figure 2 shows that APP increase leads to reduced hardness of brad texture immediately after baking, 24, 48, and 72 hours of keeping duration ($P<0.05$). This may indicate the fact that staling progressed at a slower pace in bread with APP. Therefore, 3 % treatment was reported as the slowest staling pace.

This might be associated with the fact that increased water absorption by fiber compounds

in APP and increased moisture of bread content have led to reduced hardness of bread texture (Chen et al., 1988; Rogers et al., 1988). In other words, investigating hydrocolloids showed that the large number of hydroxyl groups in the hydrocolloids of fiber structure increase the water absorption by creating hydrogen links (Rosell et al., 2001). The mechanism of hydrocolloid impact on reducing the hardness of the bread crumb can be associated with the competition between hydrocolloid and starch in absorbing water, leading to appropriate distribution of water and increased water shelf life in bread (Armero and Collar, 1996; Eidam et al., 1995). Biliaderis et al. (1997) stated that the effect of hydrocolloids on reduced bread hardness is resulted from first reduced swelling of starch granules and amylose withdrawal and second, their suppressive effect on the structure of the starch due to preventing the integration of amylose chain (Biliaderis et al., 1997).

Results also show that, at treatments 5 % and 7 %, bread hardness increased. In other words, the reaction between gluten and fiber at high levels leads to weakened gluten network and accordingly, reduced volume and increased hardness of the texture (Chen et al., 1988; Pomeranz and Shogren, 1977). These results are consistent with those of study conducted by Zhou et al. (2008) who investigated the effect of tea polysaccharides on the characteristics of breads (Zhou and wang, 2009).

The comparison of means in table 2 shows that increased APP led to significant reduction in the cohesiveness of bread after baking and declined in all treatment by passage of time ($P<0.05$). In all keeping periods except 24 hours, no significant difference is found between with APP and control sample. In other words, similar to the study conducted by Gomez et al. (2010), cohesiveness declined in effect of fiber on the cake (Gomez et al., 2009).

Table 2. Effect of APP and time on Bread cohesiveness Factor

Treatment	24h	48h	72h	96h
control	0.94 ± 0.017^{aA}	0.84 ± 0.005^{aB}	0.73 ± 0.075^{aC}	$0.75^{aC}\pm0.01$
1%	0.94 ± 0.017^{aA}	0.83 ± 0.01^{aB}	0.72 ± 0.075^{aC}	$0.75^{aC}\pm0.01$
3%	0.91 ± 0.005^{bA}	0.84 ± 0.005^{aB}	0.74 ± 0.025^{aC}	$0.75^{aC}\pm0.01$
5%	0.89 ± 0.01^{bcA}	0.83 ± 0.01^{aB}	0.75 ± 0.02^{aC}	$0.75^{aC}\pm0.01$
7%	$0.87\pm.01^{cA}$	0.84 ± 0.005^{aB}	0.75 ± 0.01^{aC}	$0.74^{aC}\pm0.01$

A, B, and C show a significant difference between these values in different days and a, b, and c shoes the significant difference in different treatments ($P<0.05$).

3.4. Image Analysis

The results of table 3 show that L (lightness) has significantly reduced in sample bread with APP ($P<0.05$). a and b factors, however, have significantly increased in samples with APP ($P<0.05$). In other words, image analysis evaluation, considering the reduced L and increased a and b, shows that samples with APP are darker than control sample. This result is consistent with that of various studies such as the effect of Cladodes from Opuntia ficus indica on cake (Ayadi and Abdelmaksoud, 2009), adding tomato pomace in Barbari bread

(Barbari bread is a type of Iranian flatbread. It is one of the thickest flat breads. It's very popular in Iran) (Majzoobi et al., 2011) and various fiber sources on biscuits (Sudha et al., 2007) due to the reaction between Millard and caramelization.

3.5. Sensory Evaluation

Table 4 shows that adding APP increases the bread aroma compared to control sample. Texture feature at 3 % significantly has the

highest score compared to other samples (P<0.05). Color characteristics results show that the highest score belongs to control sample with a significant difference. The results of taste show that, at 5 % and 7 % accounted for the lowest score (P<0.05). In other words, the results show that APP has positive effect on aroma and texture of Sangak bread. The positive effect of APP on bread texture characteristics was also reported by Majzoobi et al. (1998). Adding high level of APP to Sangak bread had negative effect of color and taste which is consistent with the results of the study conducted by Masoodi et al. (1998). Therefore, the acceptability declined (Masoodi et al., 1998).

Table 3. Comparison of Mean factors in color analysis

Treatment	L	a	b
control	83.06±0.36 [a]	2.03±0.54 [a]	22.52±1.7 [a]
1%	80.91±0.12 [b]	4.31±1.5 [b]	26.53±1.41 [ab]
3%	79.9±0.63 [bc]	5.37±0.15 [bc]	27.92±0.77 [b]
5%	79.21±1.06 [bc]	7.34±1.5 [cd]	29±3.8 [b]
7%	79.4±1.5 [c]	7.93±1.01 [d]	28.98±2.6 [b]

a, b, and c show the difference between various treatments (P<0.05)

Table 4. The effect of APP on organoleptic properties of bread

Treatment	smell	texture	taste	color	apprance	acceptability
control	2.9±0.15 [a]	3.3±0.15 [a]	3.4±0.1 [ab]	4±0.2[c]	3.5±0.1[bc]	3.5±0.1[b]
1%	3.7±0.1[c]	3.7±0.1 [b]	3.6±0.1[bc]	3.4±0.1[ab]	3.4±0.1[b]	3.6±0.1[b]
3%	3.9±0.1[c]	4.1±0.15 [c]	3.7±0.1[c]	3.5±0.15[b]	3.6±0.1[c]	3.6±0.1[b]
5%	3.2±0.1[b]	3.5±0.1 [ab]	3.3±0.1[a]	3.3±0.1[ac]	3.2±0.1[a]	3.1±0.1[a]
7%	3.4±0.1[b]	3.3±0.1 [a]	3.2±0.05[a]	3.2±0.1[a]	3.2±0.05[a]	3.2±0.1[a]

a, b, and c show the difference between various treatments (P<0.05)

4. Conclusion

Adding APP to Sangak bread improves various properties of bread including softer texture and delayed staling compared to control sample. Consumers rated the APP (up to 3 %) acceptable. High level of APP, however, reduced the acceptability due to the impact on color. APP can be introduced as an accessible, abundant source, and cheap source of fiber in order to improve the texture and to some extent the organoleptic properties of native bread (Sangak).

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EFFECT OF *THYMUS DAENENSIS* EXTRACT ON OXIDATION STABILITY AND FORMATION OF TRANS FATTY ACIDS IN FRIED PANJEREIE BREAD AND FRYING OIL

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ABSTRACT

The effect of ethanolic *Thymus daenensis* extract (TE) incorporation, as a natural antioxidant, on oxidation stability as well as the formation of trans fatty acids in used frying oil in preparation of panjereie bread (Iranian traditional cookie) was investigated. The frying was done with a mixture of soybean and palm (1:1) edible oils contained (1, 2 and 3) % TE, in comparison to TBHQ (1 %), at180 °C for 1 hour. According to related results of acid value, peroxide value, conjugated diene value, anisidine value, and total polar compounds analysis; employing the TE or synthetic antioxidant could reduce the formation of oxidation compounds in frying oil in addition to fried panjereie bread. However, by elapsing of time, radical scavenging activity decreased for all samples, the lowest scavenging activity can be correlated to the samples containing 3 % TE. The slowest kinematic viscosity was observed in samples containing 3 % TE. However, the formation of trans fatty acids was increased by elapsing the frying time, the lowest amount of trans fatty acids was observed in prepared panjereie bread with oil containing 3 % TE. Moreover, TE of (1 and 2) % and TBHQ did not have significant difference ($P<0.05$). The TE can be used as a natural antioxidant to reduce the oxidation as well as formation of trans fatty acids in frying oils and fried products.

1. Introduction

The used frying oil in frying as a thermal process for food preparation can act as an environment for transferring the heat to food. The quality of fried food should be correlated with type of food as well as used oil. Due to applied high temperature in frying also in addition to present of the air and moisture in the environment, the physical and chemical degradation process could cause adverse effects on oil performance, quality and safety of fried food (Choe and Min, 2007; Debnath et al., 2009).

However, the produced volatile compounds during the frying can be lost, non-volatile compounds might be accumulated which could stimulate the oxidation and oil degradation and consequent health risks for consumers (Choe and Min, 2007). Such non-volatile products include monomeric triglycerides and polymeric triglycerides that have cyclic fatty acyls and different breakdown products (Urbancic et al., 2014).

According to recent findings, that the correlation between the trans fatty acids and

cardiovascular disease were well investigated (Mozaffarian et al., 2006). Although in the past, trans fatty acids were attributed to oil hydrogenation, now the frying can be considered as one of the most important reasons for the forming of trans fatty acids as function of on frying conditions and the quality of used materials (Liu et al., 2008).

Strong synthetic antioxidants like TBHQ, BHT and BHA are used to prevent the oils degradation during the frying (Zhang et al., 2004).Recently, due to some raised health concerns as result of synthetic antioxidant consumption the application of natural antioxidants attracted considerable attention. One of the natural additives is herbal compounds which could play important role in food industry (Hashemi et al., 2015).

Thymus genus that is known as Avishan in Iran is one of the important aromatic plants which due to biological and pharmaceutical characteristics can be used as a medicinal plant. *T. daenensis* is one of the 4 endemic species out of 14available species in Iran which is widely used as drink and food additives (Mozaffarian, 1996).

Formation of oxidation compounds in frying oil and also in food materials during frying is important, so in this study, panjereie bread, a traditional cookie in Iran produced by the deep frying process, is used as a food model. The aim of this study is to investigate the use of *T.daenensis* extract in the stabilization of frying oil, as well as to study the formation of oxidation compounds and trans fatty acids in panjereie bread.

2. Materials and methods

2.1. Materials

Dried leaves of *T. daenensis* plant (12 % moisture) were provided by a local store in Shiraz city (Fars, Iran) in March 2016. Refined frying oil (soybean oil + palm oil) with no additives was provided by the Narges Oil Company, Shiraz, Iran. All of the chemical materials were purchased from Merck (Darmstadt, Germany) and Sigma Chemical Company (Sigma_Aldrich GmbH, Steinheim, Germany).

2.2. Preparation of ethanolic *T. daenensis* extract

T. daenensis leaves were ground to powder then mixed with ethanol in a ratio of (1:10 w/v). Furthermore, the extraction process was carried out for 48 hours at 40 °C. After filtering, the extract was concentrated with a rotary evaporator (Hahn shin, Korea) at 45 °C and stored at -18 °C until the day of experiments.

2.3. Total phenolic compounds

Phenolic content of TE was determined by Folin Ciocalteu method and absorbance of samples was measured at a wavelength of 765 nm. For preparation of the blank, distilled water (400 μL) was used instead of the sample (Kahkonen et al., 1999).

2.4. Total flavonoids

Flavonoids value in TE was determined by using colorimetric of quercetin method. Quercetin (0-500 mg/L) was used as the standard. Sample absorbance was read at 510 nm and flavonoids value was reported in terms of mg/g (Kim et al., 2003).

2.5. DPPH assay

Different concentrations of TE diluted with ethanol were mixed with 1.0 mL of a 0.3 mM DPPH ethanol solution. Samples absorbance was read at a wavelength of 517 nm after 30 minutes of reaction time (Choi et al., 2002).

2.6 Ferric reducing antioxidant power (FRAP) assay

Briefly, 900 mL FRAP reagent was mixed with 90 mL distilled water then warmed to 37 °C in a water bath. The control reading of the reagent was determined at 595 nm. Subsequently, 30 mL of sample solution (100 mg in 10 mL of n-hexane) was added and absorbance was determined at 595 nm against the control solution. $FeSO_4.7H_2O$ was used as the standard and the results were reported on the basis of mmol Fe^{2+}/g (Benzie and Strain, 1996).

2.7 Frying procedure

TE at concentrations of (1, 2 and 3) % and TBHQ antioxidants at a concentration of 1% were

added to the oil. The frying procedure took place with the help of deep-fat fryer with a 2.5-L-volume vessel at 180°C for 1 hour. Oil and panjereie bread were sampled every 5 minutes. In other words, sampling was performed 12 times for 1 hour. Oil samples were filtered and stored at 4 °C.

2.8. Oil extraction

The oil of panjereie bread was extracted with the use of chloroform/methanol (1:2, v/v) (Bligh and Dyer, 1959).

2.9. Determination of acid value

The acid value was determined according to AOCS method (1993) by titration method using potassium hydroxide.

2.10. Peroxide value (PV)

Acetic acid-chloroform oil samples were mixed with 0.5 mL saturated potassium iodide solution (5± 0.05 g) and were titrated using 0.1 N sodium thiosulphate(AOCS, 1998).

2.11. Conjugated diene value (CDV)

Oil samples were mixed with hexane and their absorbance was read at a wavelength of 234 nm (Shimada et al., 1991).

2.12. Anisidine value (AnV)

Oil samples (0.5–4 ± 0.001 g) were mixed with 1 mL p-anisidine and 25 mL isooctane and then their absorbance were read at a wavelength of 350 nm (AOCS, 1998).

2.13. Determination of radical scavenging activity

50 mg of sample oil was mixed with 4 mL DPPH dissolved in toluene (10^{-4} M) and after 60 minutes; their absorbance was read at a wavelength of 515 nm (Ramadan et al., 2006).

2.14. Determination of total polar compounds

The TPC estimation of oil samples was determined directly in the oil, with a food oil monitor testo 265 (Testo company, Germany), according to the manufacturer`s instructions.

2.15. Measurement of kinematic viscosity

The kinematic viscosity of the oil samples during frying was determined with a Cannon Ubbelohde calibrated capillary viscometer (Cannon Instrument Company, State College, Pennsylvania) by the method reported by Debnath et al. (2012).

2.16.Determination of fatty acid composition by GC-FID

Transesterification of fatty acids into their corresponding fatty acid methyl esters (FAMEs)was done by vigorous shaking of oil in hexane (0.3 g in 7 mL) with 2 mL of 7 mol/L methanolic KOH at 50 °C for 10 min The FAMEs were analyzed using an gas chromatography (GC) HP-5890 (Agilent, Palo Alto, CA, USA) equipped with a CP 88 3400 (Varian Inc., Palo Alto, CA, USA) capillary column of fused silica (120 m in length ×0.25 mm in internal diameter, 0.25µm film thickness) using an flame ionization detector (FID). The used carrier gas was helium with a flow rate of 0.8 mL/min. The oven temperature gradient was 5°C each 5 min from 160 °C to 200 °C; temperatures of the injector and the detector were adjusted as 210 °C and 300 °C, respectively(Sherazi et al. 2009; Asnaashari et al. 2015).

2.17. Statistical analysis

Statistical analysis was performed with one-way ANOVA and significant differences between samples were determined by Duncan's multiple range tests. The SPSS package program (v. 22.0 for Windows, SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. Differences were considered significant at $P<0.05$

3. Results and discussion

3.1. Extract characterization

Some of the observed antioxidants and antimicrobial characterizations in herbal extracts can be correlated to their phenolic compounds. In this context, with increasing in concentration of phenolic compounds, the antioxidants and antimicrobial will be increased (Hashemi et al.,

2015). The measured level of phenolic compounds in TE was reported as 95±2 mg/g which is relativity higher than indicated level for other plant extract such as *Matricaria recutita*(41±2.5 mg/g) (Hashemi et al., 2015)as well as lower in comparison with rosemary (112 mg/g) (Chammem et al., 2015). The flavonoids as herbal polyphenols which has been categorized in more than 4000 types can posse antioxidant and antimicrobial activities. The flavonoids amount in the TE was noted as 44±3 mg/g which is higher than explored concentration in *Matricaria recutita* (26±1.4 mg/g) (Hashemi et al., 2015).

Several factors that could affect the phenolic and flavonoids compounds concentration in herbal extracts, can be summarized as the extraction method, alterations in genetic, the grow climate and harvest conditions (Proestos et al., 2006; Chammem et al., 2015).

In the extraction approaches; although methanol, due to high polarity, is usually used as a solvent, due to observed limitations of methanol application in food industries such as toxicity, ethanol was utilized as a solvent in current study (Nor Aini et al., 2008).

Table 1. Trans fatty acids formation in extracted oil from fried panjereie bread samples during frying at 180 °C for 1 h.

	Time (min)	**cis-C18:1**	**trans-C18:1**	**cis,cis-C18:2**	**trans-C18:2**	**cis,cis,cis-C18:3**	**trans-C18:3**
Control	**0**	33.52±0.23^{g}	0.07±0^{e}	31.75±0.11^{a}	0.08±0^{e}	3.32±0.04^{a}	0.03±0^{e}
	30	34.82±0.16^{b}	0.43±0.02^{b}	30.12±0.15^{f}	0.53±0.04^{b}	2.8±0.07^{f}	0.18±0^{b}
	60	36.22±0.31^{a}	0.68±0.07^{a}	29.22±0.12^{g}	0.91±0.06^{a}	1.9±0.05^{g}	0.29±0.02^{a}
Extract-1%	**0**	33.52±0.23^{g}	0.07±0^{e}	31.75±0.11^{a}	0.08±0^{e}	3.32±0.04^{a}	0.03±0^{e}
	30	33.92±0.35^{e}	0.16±0.03^{d}	31.18±0.10^{c}	0.22±0.03^{d}	3.12±0.04^{c}	0.09±0^{d}
	60	34.62±0.15^{c}	0.38±0.05^{c}	30.59±0.13^{e}	0.51±0.05^{c}	2.71±0.03^{e}	0.15±0.02^{c}
Extract-2%	**0**	33.52±0.23^{g}	0.07±0^{e}	31.75±0.11^{a}	0.08±0^{e}	3.32±0.04^{a}	0.03±0^{e}
	30	33.82±0.31^{e}	0.15±0.03^{d}	31.10±0.12^{c}	0.20±0.04^{d}	3.19±0.06^{c}	0.08±0.02^{d}
	60	34.54±0.13^{c}	0.35±0.04^{c}	30.47±0.14^{e}	0.47±0.04^{c}	2.76±0.04^{e}	0.13±0.03^{c}
Extract-3%	**0**	33.52±0.23^{g}	0.07±0^{e}	31.75±0.11^{a}	0.08±0^{e}	3.32±0.04^{a}	0.03±0^{e}
	30	33.22±0.11^{f}	0.09±0.02^{e}	31.54±0.13^{b}	0.11±0.03^{e}	3.26±0.04^{b}	0.05±0.03^{e}
	60	34.07±0.16^{d}	0.19±0.06^{d}	30.87±0.16^{d}	0.24±0.05^{d}	2.93±0.02^{d}	0.09±0^{d}
TBHQ-1%	**0**	33.52±0.23^{g}	0.07±0^{e}	31.75±0.11^{a}	0.08±0^{e}	3.32±0.04^{a}	0.03±0^{e}
	30	33.79±0.30^{e}	0.14±0.03^{d}	31.12±0.11^{c}	0.19±0.03^{d}	3.17±0.05^{c}	0.09±0.02^{d}
	60	34.50±0.12^{c}	0.33±0.05^{c}	30.41±0.13^{e}	0.45±0.05^{c}	2.75±0.03^{e}	0.14±0.02^{c}

All values are means of three determinations. Means within a column with the same lowercase letters are not significantly different at $P<0.05$

3.2. In vitro antioxidants activity of extract

The result of DPPH assays for evaluation of antioxidant activity of TE showed that IC_{50} of the extract was 32.6±1.4 µg/mL whereas the reported IC_{50} for BHT was 18.8±0.7 µg/mL. In comparison to *Matricaria recutita* extract with IC_{50} =82.3±2.8

µg/mL, much antioxidant power by employing of TE can be obtained (Hashemi et al., 2015).

FRAP as a simple and fast method to evaluate the antioxidant activity which was reported as TE 15.85±0.47 mmol Fe^{2+}/g shows that antioxidant power of TE is very good.

The observed good characteristic of an antioxidant could be attributed to phenolic compounds in TE. Phenolic compounds in herbal extract have multi-functional characteristics and can act as reviving, oxygen radicals neutralizing and metals chelating agents. So, as a result of their activities, oxidation processes will be reduced (Su et al., 2007).

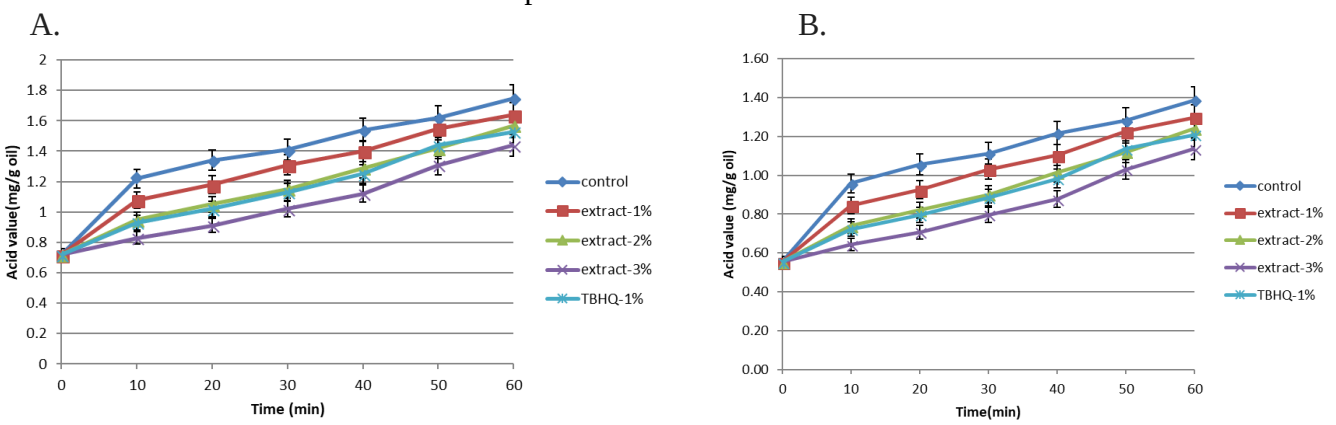


Figure 1. Acid value of fried oil samples (A) and extracted oil from fried panjereie bread samples (B) during frying at 180 °C for 1 h.

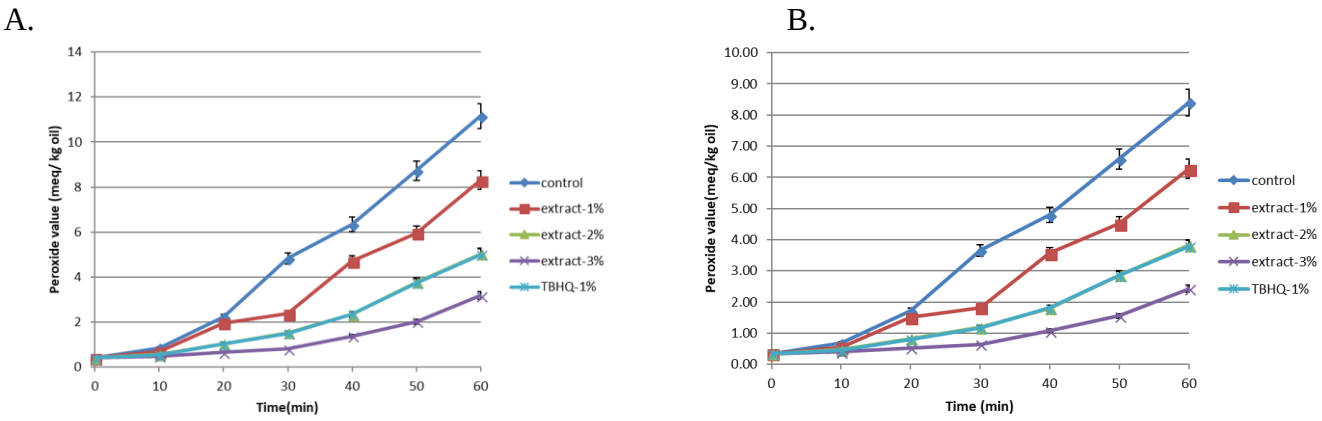


Figure 2. Peroxide value of fried oil samples (A) and extracted oil from fried panjereie bread samples (B) during frying at 180 °C for 1 h.

3.3. Antioxidant activity of TE during frying

Free fatty acids are formed during the frying process in frying oil due to the formation of free radicals and break down of double bonds. The recorded changes in fatty acid value are presented in Figure 1. In all of the samples the AV increased by passing the frying time. For example, AV reached 1.75 from the initial value of 0.72 during 1 hour in control sample. Ma et al. (2014) reported that AV for Kizakinonatane oil increases by the increment in the frying time. According to results of current investigation, the TE at concentration of

3 % showed the greatest influence in hydrolysis of fatty acids reduction. Also, by elapsing the frying time the AV increased. Ramadan et al. (2006) reported the increase in free fatty acids was due to the oxidation and cleavage of double bonds to produce carbonyl compounds, which oxidized to low molecular weight fatty acid during frying.

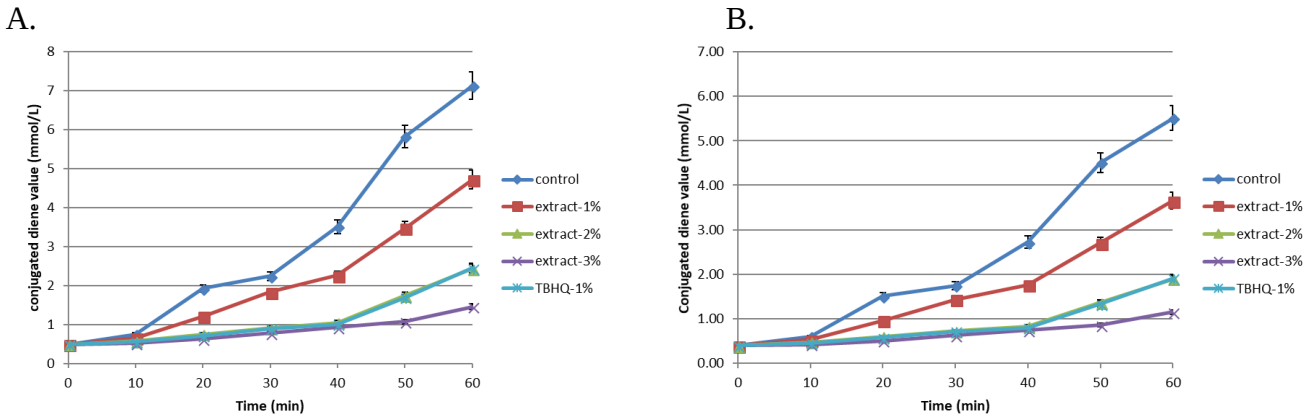


Figure 3. Conjugated diene value of fried oil samples (A) and extracted oil from fried panjereie bread samples (B) during frying at 180 °C for 1 h.

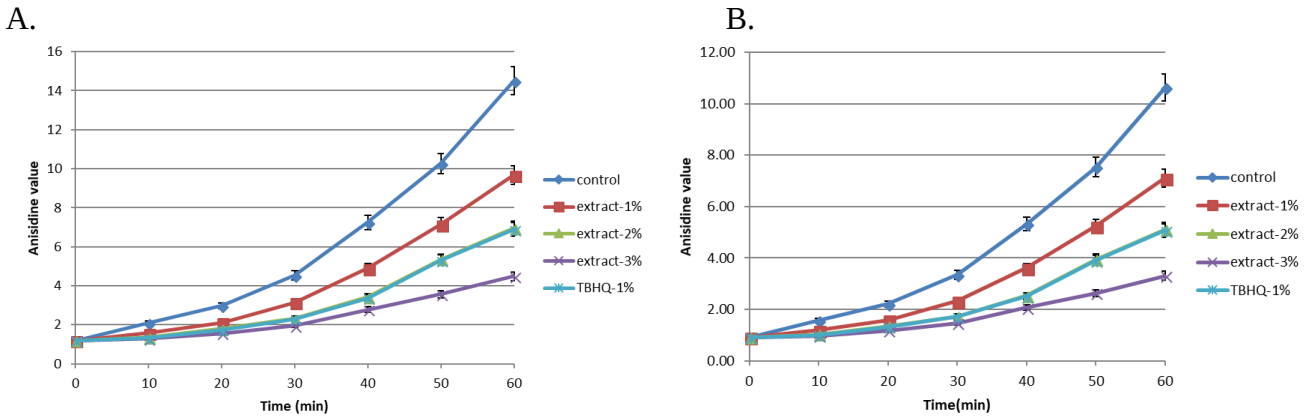


Figure 4. Anisidine value of fried oil samples (A) and extracted oil from fried panjereie bread samples (B) during frying at 180 °C for 1 h.

Antioxidant activity of TE in frying oil as well as the reduction in oxidation products in panjereie bread was investigated. Peroxide value for frying oil and panjereie bread during frying time over a period of 1 hour is presented in Figure 2. Peroxide value for frying oil increased by elapsing the frying time. The amount of hydroperoxides in oil was decreased by increasing the concentration of TE. The highest activity in reducing the concentration of hydroperoxide can be achieved with application of TE in concentration of 3%. So that formation of peroxide in panjereie bread during the frying can be reduced by incorporation of TE and TBHQ in different concentrations. Che Man and Jaswir (2000) reported that addition of rosemary extract at a concentration of 0.4 % improved the stability of palm oil and reduced peroxides formation to 37%. According to Chammem et al. (2015); as result of rosemary extract employing (0.08 %) in conducted frying using the mixture of sunflower oil and soybean oil could reduce hydroperoxides formation to 38 %.

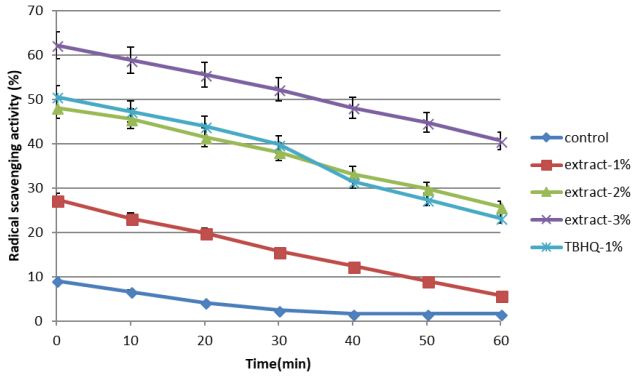
The CD measures the primary oxidation compounds. Although the CD was increased

among all of the samples during frying, the lowest level was recorded in the oil samples containing TE of 3 % (Fig.3). After frying for 12 hours, the amount of CD in control sample was higher in comparison with other. Although lower CD was noted for fried panjereie bread, in oil containing antioxidant showed, the lowest can be correlated oil containing 3 % extract. Aachary et al. (2014) reported that addition of crude canolol extract could increase the stability of canola oil during frying; and CD amount after frying was less than other samples. Urbancic et al. (2014) reported that incorporation of rosemary extract into sunflower oil reduces CD formation during frying.

Antioxidant activity of TE was also evaluated by examining the formation of secondary oxidation compounds. The results of anisidine test showed (Figure 4) that AnV was increased for all samples during the frying time. In this study, after 12-hour frying, AnV was measured as 14.51±0.36 for control samples and 4.48±0.13 for a sample containing 3% TE. No notable difference between the sample containing 2 % extract and the sample containing TBHQ was observed. AnV in panjereie bread samples fried by oil containing 3 % TE was less than other samples. Anchary et al. (2014) indicated that phenolic extracts from canola oil deodistillates and crude canolol extract, compared to BHT, had reduced anisidine amount in canola oil during frying.

RSA results showed that potential of oil samples to quenching DPPH radicals reduced during frying (Figure 5). Debnath et al. (2012) reported that RSA was reduced during frying the oil due to the decomposition of the antioxidant and/or the volatilization of antioxidant through evaporation. The extract contains phenolic compounds which can be destroyed by high frying temperature.RSA in oil sample containing 2 % TE, similar to oil sample containing TBHQ 1%, had a decreasing trend. At the end of the frying process, sample containing extract in the concentration of 3 % had the highest RSA. RSA results in samples of panjereie bread were similar to oil samples and the samples fried in oil containing antioxidant had more RSA.

A.



B.

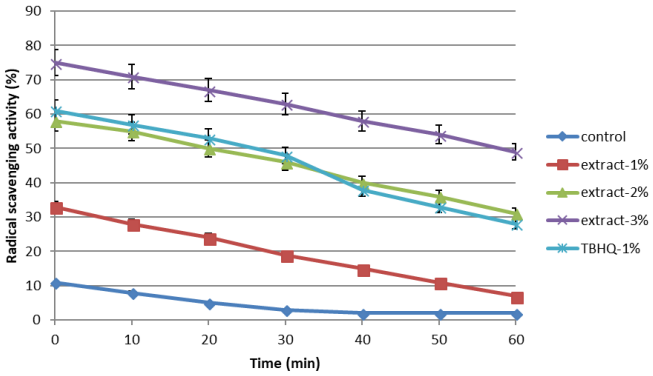


Figure 5. Radical scavenging activity of fried oil samples (A) and extracted oil from fried panjereie bread samples (B) during frying at 180 °C for 1 h.

TPC is one of the most important indicators of oil degradation during frying and includes non-volatile cyclic and polymeric compounds. In many countries, when the amount of these compounds in oil rises to 24-25%, consumption of oil is prohibited (Wai, 2007). Variations in TPC for all of the samples during frying are demonstrated in Figure 6. These results show that TPC amount increases by passing the frying time. These results are similar to Houhoula et al. (2002).TPC increase in samples were ordered from highest to lowest as control > 1% TE> 2% TE= TBHQ> 3% TE. These results suggest that the most stable formulation against TPC formation in oil was 3% TE. TPC amount in panjereie bread samples was the greatest amount among samples fried in oil without antioxidant. The existence of TE and TBHQ in frying oil reduced the formation of TPC in

panjereie bread. Casarotti and Jorge (2012) reported that when rosemary extract was used as an antioxidant in frying oil, the formation of polar compounds were reduced during frying.

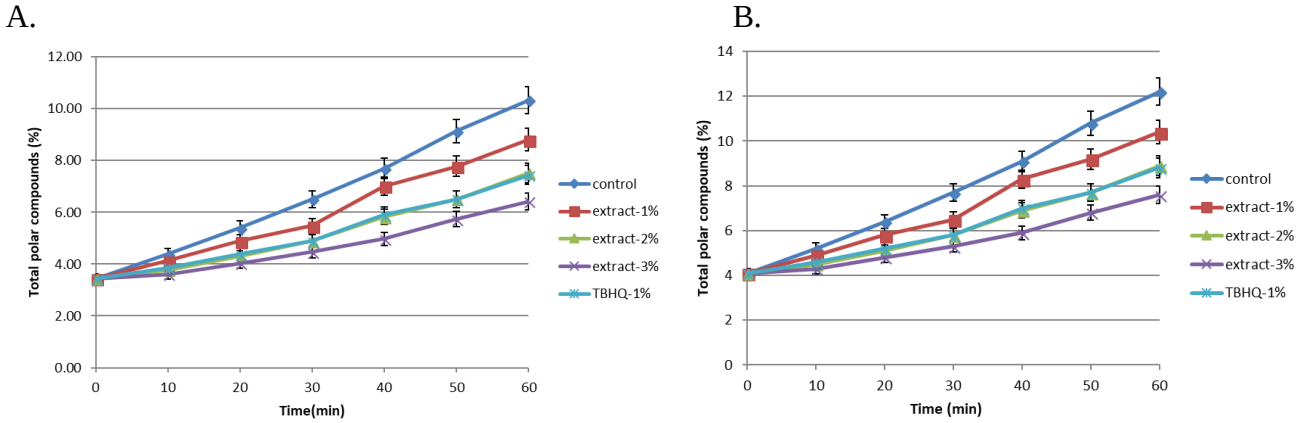


Figure 6. Total polar compounds of fried oil samples (A) and extracted oil from fried panjereie bread samples (B) during frying at 180 °C for 1 h.

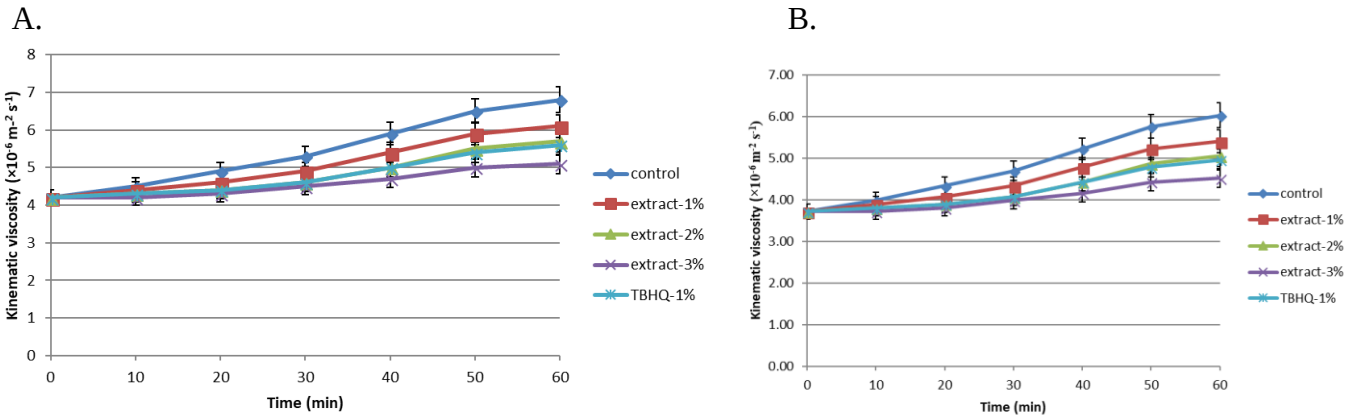


Figure 7. Kinematic viscosity of fried oil samples (A) and extracted oil from fried panjereie bread samples (B) during frying at 180 °C for 1 h.

Changes in kinematic viscosity are presented for different samples of oil during frying (Figure 7). Results showed that kinematic viscosity increased with increasing time of frying in all samples. The minimum rate of changes in kinematic viscosity was observed for oil sample containing 3% TE. Viscosity increase in frying oils is related to polymerization reactions and thermal oxidation. As a result of these reactions, high molecular weight polymeric compounds are formed which increases the viscosity of oil (Al-Harbi and Al-Kahtani, 1993). Teah (1988) reported that as a result of food frying, there is too much water transferred into the oil that will result in oil polymerization and polymeric compounds formation. Debnath et al. (2012) observed that increase in polar compounds in oil made the viscosity of oil to increase. So, there is lower viscosity in oil samples containing 3% TE due to less polar compounds and oxidation of the oil. Quite similar results were obtained in panjereie bread samples and samples containing TE, TBHQ in comparison to control sample had lower viscosity.

Trans fatty acids formation in panjereie bread during frying is showed in Table1. Generally, by increasing the time of frying, trans fatty acids

increased in all samples. Most of the isomer trans for fatty acids of samples were of C18:2 type. A number of trans C18:2 after 12-hour frying was measured 0.91±0.06 g/100g and 0.24±0.05 g/100 g for a control sample and sample fried in oil containing 3% extract, respectively. The amount of observed trans fatty acid for control sample was more than other samples. Panjereie bread samples fried in oil containing TE of (1 and 2) % and TBHQ, showed no significant difference in respect of trans fatty acids formation. Panjereie bread samples fried in oil containing 3 % TE showed the lowest level of trans fatty acids among samples. Generally, the results indicated that the extract reduces the formation of trans fatty acids and also degrades polyunsaturated fatty acids. Romero et al. (2000) reported that the formation of trans fatty acids after 20 frying cycles for extra virgin olive oil, high oleic sunflower oil, and the regular sunflower oil is less than 5 mg/g. Another study showed that by increasing frying time, the amount of trans fatty acids increased in soybean and peanut oil (Sebedio et al. 1996). Yang et al. (2014) reported that the formation of trans fatty acids in fried chicken fillets depends on the initial concentration of this type of fatty acids in frying oil and raw chicken fillet.

4. Conclusions

In this study, TE was incorporated to increase the stability of frying oil against oxidation and to reduce the formation of oxidation compounds as well as trans fatty acids. As result of phenolic compounds existence in the extract the potential to reduce hydroperoxides, secondary compounds of oxidation, polar compounds, and trans fatty acids in frying oil and fried panjereie bread was demonstrated by the TE. Further research is required to evaluate the efficiency of TE extract in combination with other conventional technologies.

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APPLICATION OF ARTIFICIAL NEURAL NETWORK (ANN) IN DRYING KINETICS ANALYSIS FOR POTATO CUBES

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ABSTRACT
In this research, drying time was analyzed in the laboratory for the drying of potatoes using a fluidized bed dryer. Drying was performed at the inlet temperatures of 45, 50 and 55 °C and the air velocity of 3.2, 6.8 and 9.1 m.s-1 and bed depth of 1.5, 2.2 and 3 cm. The effects of temperature, air velocity on drying time were investigated. The results showed that with increasing temperature and velocity, drying time and process moisture loss were reduced, and with reducing bed depth, the samples were dried more quickly. The lowest drying time was obtained at the velocity of 9.1 m/s and temperature of 55 ° C, and the maximum drying time was obtained at the velocity of 3.2 m/s and the temperature of 45 °C. With increasing depth, because of high substrate thickness, drying time increased, the lowest drying time was obtained at bed depth 1.5 cm and the maximum drying time was obtained at bed depth 3 cm. Also, the neural network of feedforward type with different neurons in the hidden layer and of the transfer function, Hyperbolic tangent sigmoid and the Levenberg-Marquardt algorithm was used with a number of different Epoch. The best result obtained with Epoch 500 and R2 = 099957 and 20 neurons in the hidden layer.

Introduction

Potato (Solanum tuberosum L.) ranks as the fourth universal food product, following rice, wheat, and maize (Yang et al., 2017). The dehydrated potato is an important food crop and is extensively used in ready-to-eat foods. Food problem arises in most developing countries mainly due to the inability to preserve food surpluses rather than due to low production(Ruhanian and Movagharnejad, 2016). Potato categorized among the products with a broad area under cultivation in the world and ranks in fifth place in terms of production. This glandular product not only is rich in hydrocarbons but also is an important source of minerals and vitamins. In developed countries, about 500 million people consume potatoes(Folgado et al., 2013). Noting that the potato is one of the most important agricultural crops and can have many losses due to inappropriate processing, thus drying of this product leads to increase shelf life(Roustapour et al., 2014). Given that huge amount of potatoes are produced in industrial products and are divided into two categories:1- Dried products potatoes of cooked potatoes 2- dried potato products of Fresh potatoes(Lecture and Science, 1987). Potato is one of the products, which is highly wasted in developing countries because of poor harvest and storage methods, therefore, the production of dried potato products may be necessary to increase their shelf time, and decrease wastage. Of course, other facilities, such as proper packaging, appropriate shipping, and sufficient processing may also lower a number of the wasted products (Simal et al., 1997). Preservation of food through drying is

one of the oldest and the most widespread method that can be used to enhance the strength of the food. Drying food is removing the moisture so that the product can be stored for a long time and be protected against corruption(Min et al., 2005). Drying by reducing the microbial enzyme activity and reducing the speed of chemical reactions, increases the shelf life of the product and by reducing the weight and volume of materials, packaging facilitates transportation and storage of products and decreases the cost of these procedures. In the case of drying, in addition to preventing the loss, the marketing can be controlled at sensitive times and required potatoes of a lot of consumers (such as barracks, restaurants, etc.) can be delivered as dried form(Mwithiga and Olwal, 2005). Newsday, the fluidized bed over the world for various processes are used in various industries, due to their high capacity and ability easy control heat transfer, fluid bed systems are widely used in the combustion process(Karagüzel et al., 2012). Also considering the thermal efficiency of the drying process, fluidized bed dryers—with their high heat and mass transfer and drying rates—are widely utilized in food dryings. Moreover, fluidized bed dryers have many applications in the chemistry, metallurgy, and pharmaceutical industries(Białobrzewski et al., 2008). Drying the floating bed method compared to other methods of drying, it has many advantages, which includes high intensity drying, high thermal efficiency, uniformity of drying, precise temperature control in the context and also short drying time due to have high rates of heat and mass transfer (Topuz et al., 2004). Considering that the drying is a complex process heat and mass transfer between the product and its surroundings(Sarker et al., 2015). Given that there are 200 types of dryers and for each dryer, be determines various processes such as the drying chamber temperature, pressure, air velocity (if the air carrier gas), relative humidity and time spent with regard to food products, manufacturing, purpose, and method. Drying is an energy-consuming process; given that the energy consumption is 10%-15% of total energy consumption in all industries in developed countries, the engineering features have a lot of importance in the drying processes(Motevali and Minaei, 2012). The role of the dryer is, providing the product with a more temperature from environmental conditions as well as sufficiently moisture vapor pressure in product for increasing displacement of moisture from inside product(Sarker et al., 2015). Dynamical models, the drying process is including mass transfer and heat that modeling have such processes for determining the moisture content changes after the specified time is difficult and time-consuming. Nowadays with the rapid development of computer processing technology and related software, from artificial intelligence technology be used to solve problems related to modeling and prediction process one of the methods artificial intelligence started on a wide scale in the drying from the last decade For simulating and predict required parameters is developing in the drying processes, are artificial neural networks , In this study, from Artificial neural networks can be used for process modeling(Farkas et al., 2000). Intelligent methods basic is the use of test inherent data that attempts to extract the intrinsic relationship between them is in other situations(Dayhoff et al., 1984). Artificial neural networks are an adaptation of biological neural networks and have been created based on biological neural networks work. In biological systems is a neuron that has different parts and in neurons, entering data is parallel and this is a distinction between biological neural systems and computer systems. In a biological system, billions of neurons are interconnected to form a dense mass That each will act as a small processor(Sablani and Rahman, 2003). In research conducted by researchers, Kashani Nejad et al. 2009 Multilayer Perceptron network (MLP) neural network and radial basis function (RBF) to estimate grain moisture were used during soaking. Artificial neural networks to model soaking wheat seeds were used at different temperatures and a comparison with the results obtained from the model page was created. Soaking temperature and time were

used as input parameters and relative humidity is used as an output parameter. The MLP neural network model is used for describing the features found in wheat seed soaking(Kashaninejad et al., 2009). Amiri Chaijan et al. The estimated conversion factor rice using Artificial Neural Networks in fluidized bed dryer came to the conclusion the network topology after the release of 7-13-7-1 and algorithm Levenberg-Marquardt and function verge of can be Tangent-Sigmoid Function can conversion efficiency unpolished into white rice by a determine factor of 48.95 percent and mean absolute error of 0.190 predicted in different situations fluidized bed drying in the range. The results also showed that the intake air temperature, the moisture content of the unpolished, the greatest impact on conversion ratio unpolished with white rice in a fluidized bed dryer. Evaluating Kinetics Drying and modeling drying process under different conditions, in order to design an appropriate dryer, Seems essential.(R Amiri Chayjan, 2011).

In this study, we tried to evaluate kinetics drying of potatoes cubes under various temperature ranges and different levels of air velocity as well as under different treatments applied to accurate and complete results can be achieved, the model will be the experimental data obtained from experiments with artificial neural C.

2. Materials and methods

2.1. Material preparation

Freshly harvested potatoes were purchased from a local market and stored in a laboratory refrigerator at 5℃. At the beginning of each test, potatoes were washed, peeled, and cut manually using the cubic device with dimensions of 0.6× 0.6 cm and a height of 0.5 cm. The drying experiment was conducted using a laboratory fluidized bed dryer made in the Department of Mechanical Bio-systems of the University of Agricultural Sciences and Natural Resources of Gorgan, Iran.

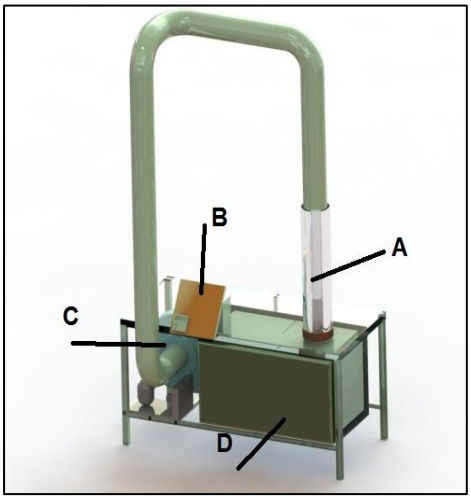

Figure 1. Schematic illustration of testing apparatus
A- Fluidizing chamber B- heater control C- fan D- heaters

2.2. Experimental procedure

To supply the required air flow, a centrifugal blower with a 3hp CDF90L_2 three-phase electric motor (KAIJIELI) was used. To measure dryer wind speed, an anemometer (LUTRON, AM-2416) with an accuracy of 0.1m/s was utilized. The dryer contained an automated temperature controller with an accuracy of ±1℃ (Fig. 1). Samples were weighed every 5 minutes using a Dj 2000A weigh scale (Shinko electric scale), which had an accuracy of 0.01 g. Samples were weighed at the beginning, and after the dryer reached to the desired temperature,

potatoes were placed inside the drying cabinet. The experiment was performed at temperatures of 45, 50, and 55℃, bed depth of 1.5, 2.2 and 3 cm and speeds of 3.2, 6.8, and 9.1 m s^{-1}. The potatoes had a cubical shape with dimensions of 0.6× 0.6 cm and a height of 0.5 cm. Each treatment was repeated three times and the test was performed at a temperature of 30℃ with a relative moisture content of 50%. Also, the moisture content potatoes were calculated using the formula (1) (Erenturk et al., 2004).

$$MR = \frac{M - Me}{Mo - Me} \quad (1)$$

M = Moisture masses

Me = Equilibrium moisture content

Mo = Initial moisture masses

2.3. Modeling Artificial Neural Network

Data before entering the network were normalized in the range of -1 to 1. Then the data was cluttered untile data equal chance for exposure in train or test. In this paper, 80% of data was applied for training and 20% of data was used to test network. Neural Network Toolbox of software Matlab R2014 (8.3.0.532) from two-layer feedforward network with 20 neurons in the hidden layer and Levenberg-Marquardt algorithm with hyperbolic tangent sigmoid transfer function was used to predict the parameters of drying time for drying of potatoes (Equation 2). and finally statistical parameters calculated including R^2, Root Mean Square Error (RMSE) and Mean Absolute Error (MAE) have been shown and related functions have been presented in equations (3), (4) and (5) (Azadbakht et al., 2107).

$$\text{Tan} - \text{sigm} = \frac{2}{[1+e^{-2x}]} - 1 \quad (2)$$

$$\text{R2} = 1 - \frac{\sum_{i=1}^{n}[P_i - O_i]^2}{[P_i - O]^2} \quad (3)$$

$$\text{RMSE} = \sqrt{\sum_{i=1}^{n}\frac{[P_i - O_i]^2}{n}} \quad (4)$$

$$\text{MAE} = \frac{\sum_{i=1}^{n}|P_i - O_i|}{n} \quad (5)$$

Where, in equations (2), (3) and (4) involving predicted values (Pi) and actual values (Oi) and the average value of data (O).

3. Results and discussions

3.1. Effect air velocity on drying time

Figures 2, 3 and 4 show effect air velocity on drying time that the highest and lowest drying time is 5400s and 3300s at speed of 3.2 m/s and 9.1 m/s, respectively. According to this Figures, the drying time has been reduced by reducing air velocity. Increased speed led to a faster reduction of particle size and created a larger area for heat and mass transmission, so much more energy was used in the drying chamber to evaporate at the beginning of drying process. That is similar to results Afshari et al (2013) on Mazafati variety in a Cabinet drier and Ayyobi et al (2014) on its grapes and raisins drying in a thin layer and parshokohi et al (2008) in the process white grapes are drying(Ayoubi. et al., 2015)(Gholami and Rashidi, 2009)(Afshari Jouybari et al., 2013).

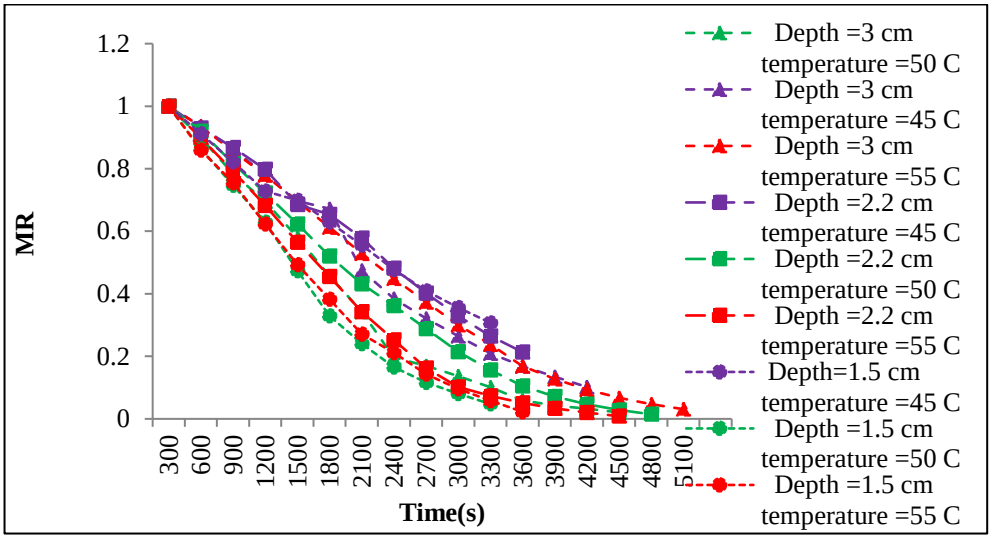

Figure 2. Diagram of drying kinetics at the speed of 3.2 m/s

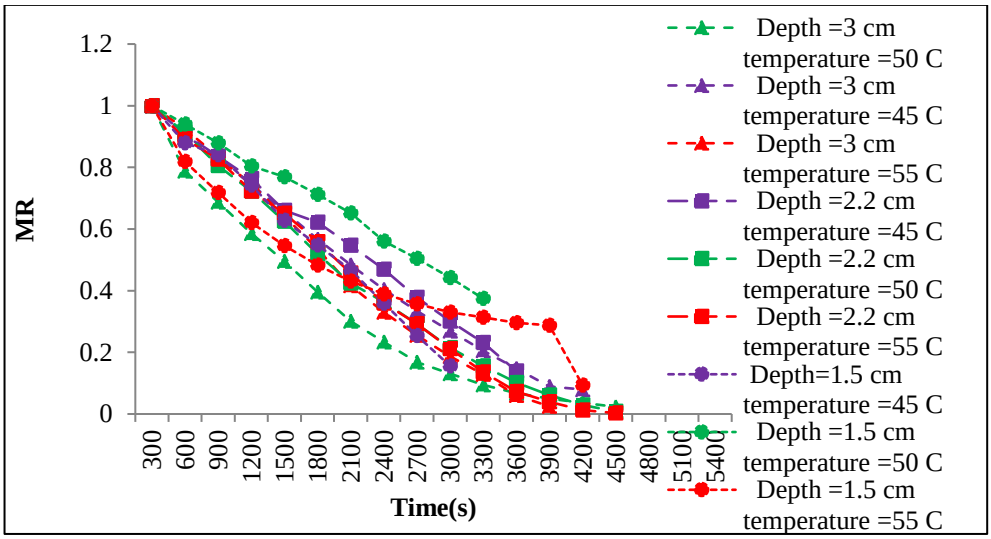

Figure 3. Diagram of drying kinetics at the speed of 6.8 m/s

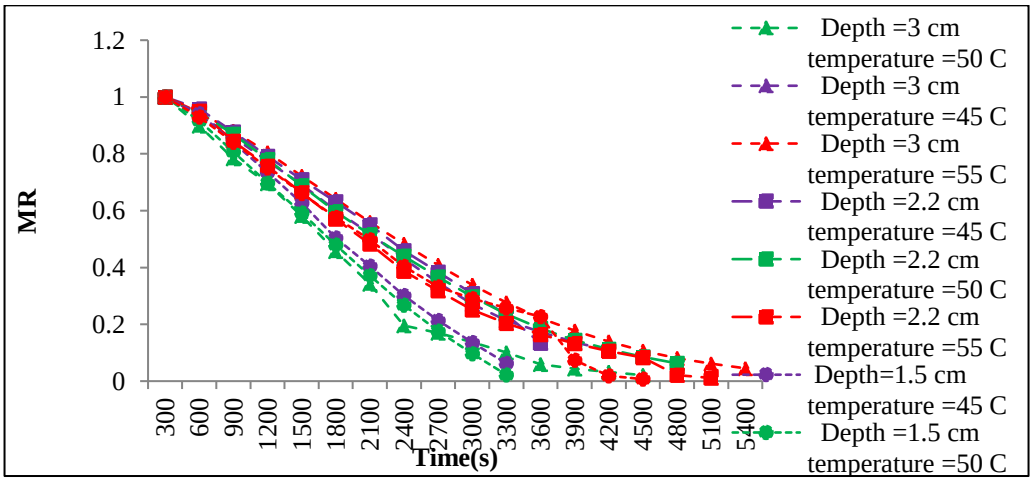

Figure 4. Diagram of drying kinetics at the speed of 9.1 m/s

3.2. Effect Depth on drying time

Figures 5, 6 and 7 shows effect depth on drying time that the highest and lowest drying time is 5400s and 3300s at depth of 3 cm and 1.5 cm, respectively. Due to increasing the layers in bed dryer and this increases the volume of the chamber, which makes the moisture out hard from the masses as well as increases the amount of energy required and drying time. Also, Azadbakht et al., (2107) concluded, in a constant depth, energy consumption would be high with increasing temperature since higher temperatures cause more moisture losses. In other words, higher temperatures lead to a further reduction of mass and moisture. That is similar to results Gazor et al. (2005) in dryer Fund on pistachio drying.(Gazor et al., 2005)(AhmadiChenarbon et al., 2011)

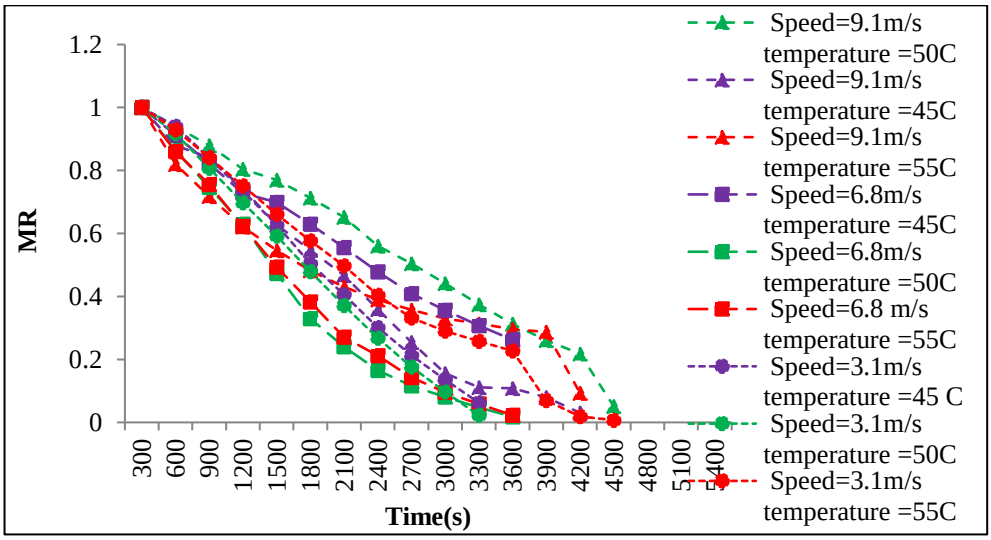

Figure 5. Diagram of drying kinetics at Depth of 1.5 cm

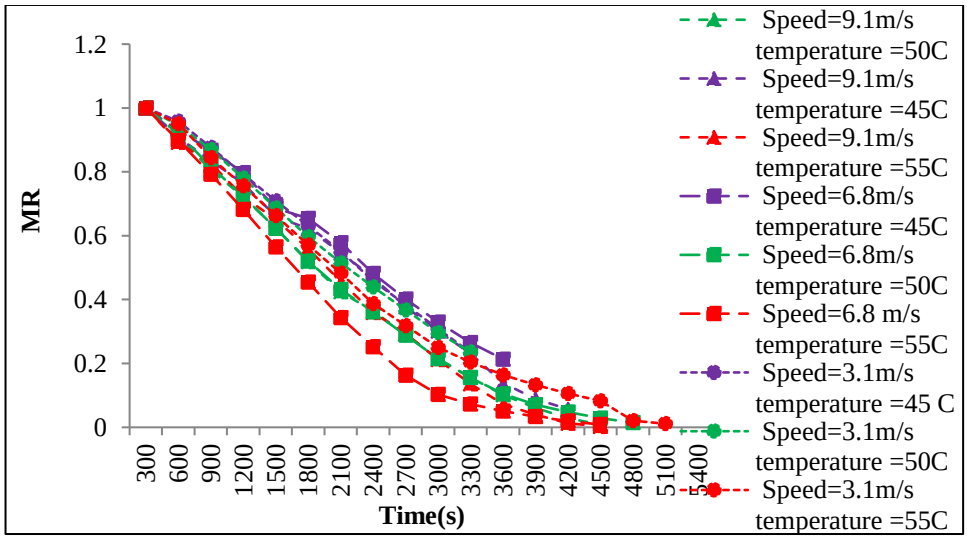

Figure 6. Diagram of drying kinetics at Depth of 2.2 cm

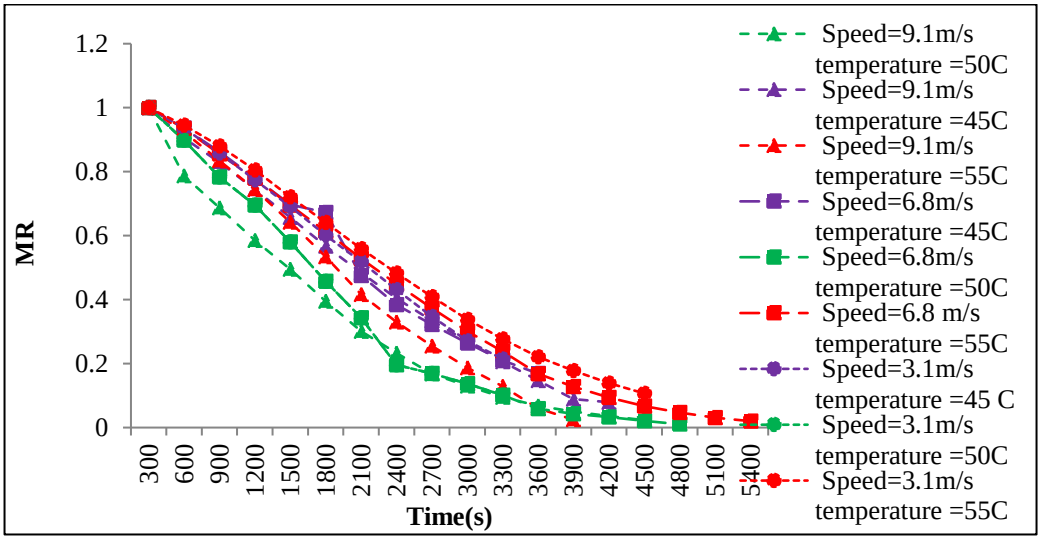

Figure 7. Diagram of drying kinetics at Depth of 3 cm

3.3. Effect temperature on drying time

Figures 8, 9 and 10 shows effect temperature on drying time that the highest and lowest drying time is 5400s and 3300s at a temperature of 45°C and 55°C, respectively. This can be explained in this way that the difference between input and output temperature of the chamber in dryer increases at first, which leads to more evaporation of water from the product. Naderi Parizi et al (2016) in a new intelligent rotary dryer under vacuum on pistachio (Kalleh Ghoochi v.) reported the same results(Naderi Parizi et al., 2016).

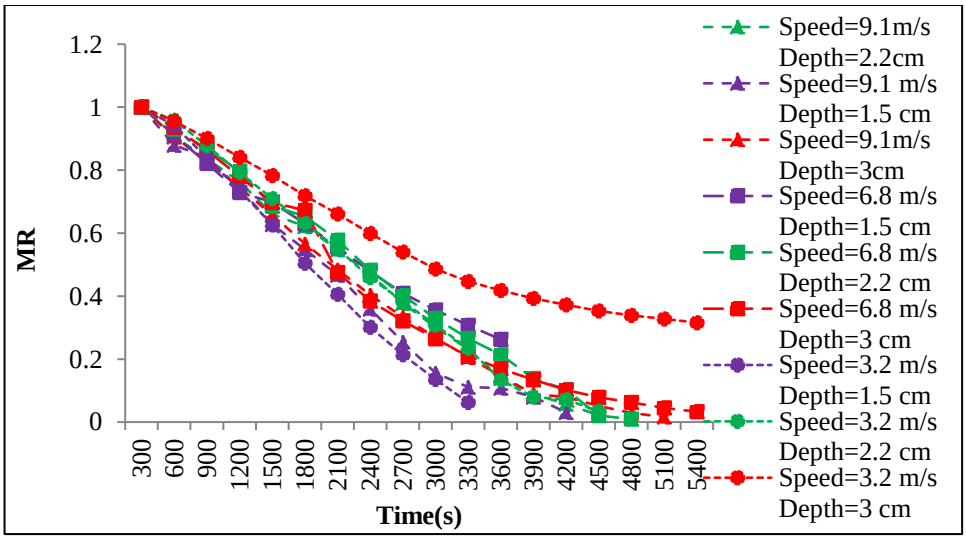

Figure 8. Diagram of drying kinetics at temperature of 45 C

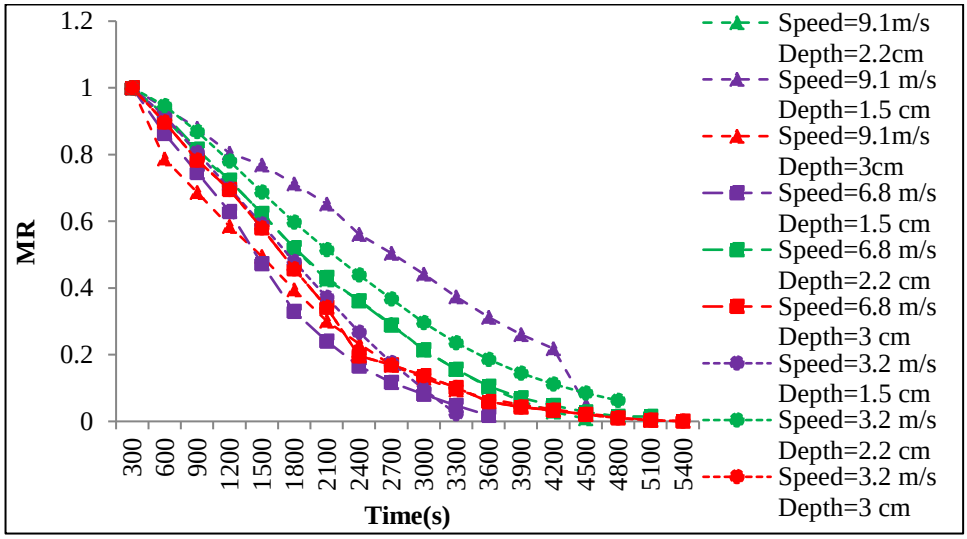

Figure 9. Diagram of drying kinetics at temperature of 50 C

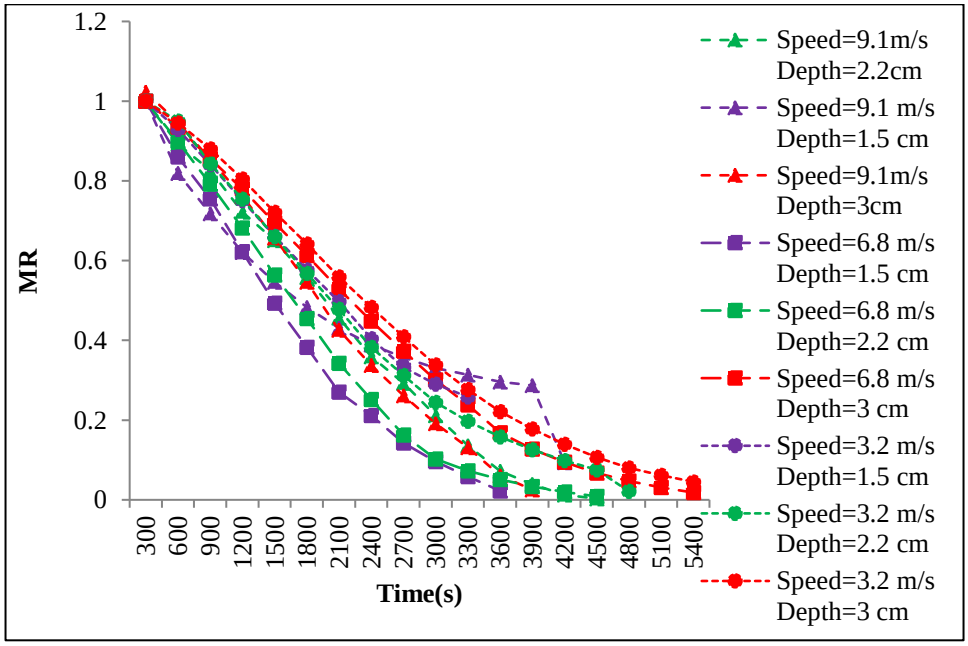

Figure 10. Diagram of drying kinetics at temperature of 55 C

3.4. Artificial Neural Network

During the modeling, process Epoch was tested the number of different neurons in the hidden layer, Up to be selected optimal topology. According to the results obtained if the criteria for suitability of a model to be the R^2, Choose a network with 20 neurons and 500 Epoch can bring the best out. Given the general trend is observed values of statistical parameters that the choice of 20 neurons has provided in the hidden layer, the complexity required for predicting moisture content in the drying process. According to the results presented in Table 1, is observed 20 neurons in the hidden layer and the number of Epoch 500 optimum values for the network, This means that increased neurons in this case better way to increase the efficiency of the neural network. Also shown in Table 2

Epoch 1000 and 20 neurons network with best results and In Table 3, this structure has created the best out. Each of these topologies can be, given the circumstances to be used for predicting relative humidity, while overall conclusion is that with increased neurons in the network structure will be achieved better results

Table 1. R^2 values in different values neurons and Epoch

Epoch	Neuron			
	5	10	15	20
250	0.97444	0.98078	0.99862	0.99402
500	0.96938	0.98679	0.99444	0.99957
750	0.97705	0.99225	0.99292	0.98997
1000	0.97795	0.99986	0.98923	0.98694

Table 2. MSE values in different values neurons and Epoch

Epoch	Neuron			
	5	10	15	20
250	0.0317	0.0135	0.0851	0.052
500	0.019	0.0210	0.041	0.0352
750	0.0315	0.0678	0.044	0.0272
1000	0.0234	0.0732	0.0206	0.012

Table 3. MAE values in different values neurons and Epoch

Epoch	Neuron			
	5	10	15	20
250	0.1128	0.0755	0.1074	0.1067
500	0.1077	0.0594	0.0666	0.0667
750	0.1029	0.0859	0.0918	0.0811
1000	0.1020	0.1007	0.0545	0.0512

4. Conclusions

According to experiments conducted by increasing the temperature and input current speed has been reduced drying time. The lowest drying time was obtained at velocity of 9.1 m/s and temperature of 55 ° C, and the maximum drying time was obtained at velocity of 3.2 m/s and the temperature of 45 °C. With increasing depth, because of high substrate thickness increased drying time the lowest drying time was obtained at bed depth 1.5 cm and the maximum drying time was obtained at bed depth 3 cm. Excessive drying leads to high energy consumption and is reduced product quality dried. Considering limitations in technology continuous measurement of moisture content and as well as dissatisfaction with its accuracy and the lack of appropriate accuracy physical models based on parameters

of dry air, in the measurement of moisture content in nonlinear processes and long lead time to process, Causing of artificial intelligence methods, especially artificial neural network used in the drying process control. Considering a survey conducted, mathematical models for process optimization and prediction of moisture content based on drying parameters is lack the tools for their application control system structures, therefore can be beneficial neural networks in the design and selection of optimal working conditions and control of the dryer. Regression models merely are not so complicated mathematical relationship, that only shows drying rate as a function of time-based on a series of coefficients and can never be a complex nonlinear relationship between variables incoming and outgoing does not specify in complex processes such as drying food. Based on the results study can the neural model be presented as an acceptable model for predict the drying kinetics of agricultural production introduced that process affected different variables and complicated, this model can, in addition to creating complex nonlinear relationships between input and output variables, determines all interactions between input variables. In general can be a lot more confidently than regression models to predict these models rely on and of this model used to optimize and control the drying process that could eventually lead to a reduction in energy consumption and drying time.

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EFFECT OF DRYING METHODS ON THE YIELD, PHYTOCHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITIES OF POTATO (*SOLANUM TUBEROSUM*) AND TWO SWEET POTATO (*IPOMOEA BATATAS*) VARIETIES

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ABSTRACT

In various parts of the world, drying technologies have been developed to convert tubers into flour which can be used as ingredients in numerous food products. Potato and sweet potato are known to contain certain bioactive constituents that are capable of scavenging free radicals and reducing the risk of degenerative diseases. The effects of two drying methods (sun and solar) on flour yield, antioxidant activity and phytochemical composition of Irish potato (IP) and two sweet potato varieties (orange and white-fleshed) were evaluated. The flour yield (15.20–19.0%) varied significantly ($p<0.05$) among the samples. The DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging activities of the flour samples varied with drying methods and the extracts' concentrations. The total antioxidant activities expressed in terms of the % inhibition (53.58–90.62%) and EC_{50} values (0.584–45.214 mg/ml) were observed to be significantly affected ($p<0.05$) by the drying methods. Drying processes generally increased the total phenolics (18.72–21.60 mg GAE/g) and total flavonoids (0.50–1.15 mg QE/g) contents of the tubers. The effect of sun-drying was observed to be less favorable on the white-fleshed sweet potato (WFSP) samples compared to solar-drying method. Although solar-dried orange-fleshed sweet potato (OFSP) flour had the highest total antioxidant activity, sun-drying method produced IP and OFSP flours with the highest total phenolics and total flavonoids contents respectively. Sun and solar dried OFSP had the highest beta-carotene content (BCC) and only the BCC of Irish potato significantly varied ($p<0.05$) with drying methods. This study also revealed that the effects of drying methods on the yield, phytochemical and antioxidant activities of the samples were mostly tuber (potato and sweet potato) dependent.

1. Introduction

Recently, foods that possess certain beneficial health effects have attracted the interest of both the researchers and the general populace. Antioxidant activity of plant materials has been attributed to varieties of phytochemicals such as alpha-tocopherol, ascorbic acid, beta-carotene phenolics, carotenoids and flavonoids (Cao *et al*., 1996; Kaur and Kapoor, 2002; Kalt, 2005). Most of these phytochemicals have

been shown to possess antimicrobial, antimutagenic and free radical scavenging activities (Friedman, 1997). Chronic illnesses and pathological disorders such as cardiovascular diseases, cancer, cataracts, neurological dysfunctions, deficiencies in immune response, age-related problems and inflammation have been attributed to free radicals including superoxide, peroxides, oxide and hydroxide ion induced oxidative stress (Diaz *et al*., 1997; Gomes *et al*., 2003). Foods that are rich in natural antioxidants are capable of neutralizing a free radical-mediated oxidative reaction through synergistic effects of health promoting compounds and offer beneficial activities in protecting human body from various diseases (Havsteen, 2002).

Potato (*Solanum tuberosum* L.) also known as Irish potato is a starchy, tuberous crop from the perennial nightshade, Solanaceae. Potato is the fourth most important food crop consumed worldwide after maize, wheat and rice. Potato is a rich source of dietary starch, proteins, fibre, minerals, vitamins and important antioxidants such as phenolic acids, carotenoids and flavonoids (Kolasa, 1993; Andre *et al*., 2007). Hesam *et al*. (2012) have shown that the methanolic extracts of potato could inhibit oxidation process due to the antiradical activity of phenolic compounds.

Sweet potato (*Ipomoea batatas* L.) of the Convolvulaceae family is an important staple crop that grows in many regions of the world. Sweet potato cultivars are known to be rich in dietary fibre, minerals, vitamins and antioxidants, including anthocyanins, phenolic acids, beta-carotene and tocopherol (Van Jaarsveld *et al*., 2006; Yildirim *et al*., 2011). Consumption of sweet potato can prevent the growth of human colon, leukemia, stomach cancer cells and ameliorate diabetes in humans (Kurata *et al*., 2007; Ludvik *et al*., 2008). Sweet potato can be grouped into different cultivars depending on their flesh and root skin colours (Woolfe, 1992). Whole white and purple skinned sweet potato are of high nutraceutical value due to high phytochemical content, radical scavenging activities, phenolic composition, essential minerals and amylose-amylopectin ratio in their peels (Salawu *et al*., 2015). Orange-fleshed sweet potato (OFSP), a bio-fortified sweet potato cultivar, has recently been of interest due to its potential as vitamin A and energy source (Hotz *et al*., 2012). OFSP has high polyphenolics, carotenoids and antioxidant contents which are mainly responsible for its health promoting function after consumption (Koala *et al*., 2013; Ruttarattanamongkol *et al*., 2015).

Seasonality of many foods coupled with malnutrition, including health and social problems impede economic progress in Nigeria and many other developing countries. Drying is commercially and economically accepted to preserve many raw and intermediate food products by reducing its bulk weight (Zhang *et al*., 2006). Although dehydration is probably the oldest method of food preservation practiced by human (Antonio *et al*., 2008), efficient drying techniques and methods that could minimize nutrient loss and retain the health potentials of food products are of utmost important. Therefore, this study aimed at determining the effect of two drying methods (sun and solar drying) on the flour yield, phytochemical composition and antioxidant activities of Irish potato and sweet potato varieties (orange and white-fleshed).

2. Materials and methods

2.1. Sample Collection

About 50 kg each of the fresh tuber samples were obtained within Ilorin metropolis, Kwara state, Nigeria. Orange-fleshed sweet potato (OFSP) was procured

from a farm in Agbamu town while Irish potato (IP) and white-fleshed sweet potato (WFSP) were purchased from Ipata and Ganmo markets respectively. The fresh tuber samples were brought to the Department of Home Economics and Food Science at University of Ilorin, Kwara State, Nigeria for processing.

2.2. Sample Preparation

The tuber samples were washed with clean tap water to remove adhering sand and dirt, peeled using a local potato peeler fabricated by Nigeria Centre for Agricultural Mechanized (NCAM), Ilorin, Kwara state and sliced to about 3.5 mm thickness using a sharp stainless steel knife. The slices were submerged under water for proper rinsing and to slow down the rate of enzymatic reactions. Each of the sliced tuber samples were divided into three portions.

2.2.1. Raw (control)

The first portions (100 g each) were ground using laboratory mortar and pestle and then subjected to phytochemical analysis.

2.2.2. Drying Processes

The second and the third portions (24 kg each) were weighed and subjected to sun and solar drying respectively as described by Kolawole *et al*. (2016). The dried samples were milled, sieved and packaged in cellophane bags (Kolawole *et al*. 2016).

2.3. Determination of OFSP, WFSP and IP Yields

The flour yields of dried OFSP, WFSP and IP were obtained by gravimetric method. The percentage yields of the samples were calculated based on dry weight as:

$$\text{Yield (\%)} = \frac{W_2}{W_1} \text{ x } 100$$

Where, W_1 = Weight of fresh sample; W_2 = Weight of the dried sample.

2.4. Extraction of Samples for Antioxidant and Phytochemical Analyses

The flour samples were extracted according to the method described by *Salawu et al*. (2015) with little modifications. Each flour sample (3 g) was extracted with 30 ml acidified methanol (1% conc. HCl in methanol) using a three steps approach as follows: A 10 ml solvent was added to each flour sample in a conical flask and completely covered with aluminum foil. The samples were stirred for 2 hrs using magnetic stirrer, centrifuged in a 50 ml plastic centrifuged tube at 1900 rpm for 10 minutes at room temperature and decanted. The supernatant and the residue was re-extracted in 10 ml of the solvent for 15 minutes, centrifuged and decanted. The supernatant was kept again and the process was repeated the third time for 20 minutes. The supernatant was combined and stored in a glass bottle covered with aluminum foil and kept at 4 ^{0}C in refrigerator until further analysis.

2.5. Antioxidant Activity (DPPH Assay)

The method described by Turkmen *et al*. (2005) with some modifications was employed to determine the DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging activity of the flour samples. Sample solutions with concentrations 10, 20, 30, 40 and 50 mg/mL were prepared from the stock solution. A 1 ml of varying concentrations of the methanolic extract solution of each flour sample was mixed and vigorously shaken with 4 ml of freshly prepared 0.1 mol/L methanolic solution of DPPH. A blank probe (control) was obtained by mixing 4 ml of 0.1 mol/L methanolic solution of DPPH and 200 µml of deionized distilled water (ddH_2O). After 30 minutes of incubation in the dark at room temperature, the absorbance was read at 517 nm against the prepared blank. The

percentage inhibition of free radicals by DPPH was calculated using the formula:

[1-(absorbance of sample/absorbance of control)] × 100.

The efficiency concentration of each sample at 50% inhibition (EC_{50}) was determined by interpolation.

2.6. Determination of Total Phenolics

The total phenolics content of methanol extract of the flour samples was determined according to the Folin-Ciocalteau method described by Singleton *et al.* (1999).

2.7. Determination of Total Flavonoids

Total flavonoids content was determined using the aluminum chloride method reported by Kale *et al*. (2010).

2.8. Beta-carotene Estimation

The method described by Kumara *et al*. (2011) was used for the β-carotene determination.

2.9. Statistical Analysis

The experiments were conducted in triplicates. All data were analyzed by one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS, version 20.0). The means were separated using Duncan's multiple range test in which $p < 0.05$ was considered to be statistically significant.

3. Results and discussion

3.1. Flour Yields of Potato, White-fleshed and Orange-fleshed Sweet potato Samples

The flour yields of potato, white and orange-fleshed sweet potato samples on dry weight basis are shown in Table 1. The drying yield which is majorly a function of the initial moisture content varied significantly ($p<0.05$) among the samples. This implied that the drying methods employed as well as the varietal (WFSP and OFSP) and tuber differences (Irish potato and sweet potato) had significant effect ($p<0.05$) on the yields of the samples. The flour yields generally ranged from 15.20% in the sun-dried potato sample to 19.00% in the sun-dried white-fleshed sweet potato sample. The flour yields obtained in this study were observed to be lower than the value of 19.60% reported by Kaur and Aggarwal (2015) for water and steam cooked potato tubers. This variation may be attributed to differences in the processing methods employed. Surprisingly, the yield of the solar dried Irish potato sample was observed to be significantly higher ($p<0.05$) than its corresponding sun dried sample while the reverse was noticed in OFSP and WFSP samples.

3.2. Total Antioxidant Activities of WFSP, OFSP AND IP Flour

The stable free radical DPPH has been widely employed to determine the free radical- scavenging ability of various dietary antioxidants by using the decrease in its absorbance at 517 nm to estimate the reduction capability of DPPH (Brand-Williams *et al*., 1995). The DPPH free radical scavenging activity of the flours increased with increasing concentration of the extracts (Figure 1). Similar trend had been reported by Hesam *et al*. (2012) for the DPPH free radical scavenging activity of three potato extracts. Variation in the radical scavenging activity of the flour extracts as a result of drying methods was observed.

The total antioxidant activities of WFSP, OFSP and IP flour extracts expressed in terms of percentage inhibition and EC_{50} are presented in Table 2. EC_{50} is a measure of efficiency concentration at which radical scavenging activity is 50% and a lower EC_{50} is an indication of higher antioxidant activity of the sample (Hesam *et al*., 2012; Irondi *et al*., 2013). It was observed that drying methods had significant effect ($p<0.05$) on the antioxidant activities (inhibitory action

and EC_{50}) of the flours. Irondi *et al*. (2013) had also reported significant effects of drying methods on the antioxidant activities of *C. papaya* seeds. The inhibitory activities and the EC_{50} values of the flour samples ranged from 53.58–90.62% and 0.584–45.214 mg/ml dry sample respectively. Many researchers had reported comparable results for the antioxidant activities of potato cultivars (91.36–94.10%), sweet potato variety (85.28%), heat treated sweet potatoes (57.89–97.92%), white and yellow-fleshed potato cultivars (65.20–89.20) (Al-Saikhan *et al*. 1995; Hesam *et al*. 2012; Tokusoglu and Yildirimi, 2012; Eleazu and Ironua, 2013). However, the relatively lower inhibitory activity of the sun-dried WFSP may be attributed to inability of the sun energy to effectively breakdown the cell structures of the sweet potato flesh to facilitate easy extraction of the antioxidants compounds. The combination of the inhibitory actions and the EC_{50} values can be used for the comparison of the total antioxidant activities of the flour samples. Hence, the order of the total antioxidant activities was solar-dried OFSP > sun-dried IP > solar-dried WFSP > solar dried IP > sun-dried OFSP > sun dried WFSP. Interestingly, the effect of drying methods on the total antioxidant activities of the flour extracts was observed to be tuber dependent. This was because the total antioxidant activities of solar-dried sweet potato varieties (WFSP and OFSP) flours were higher while that of solar-dried IP flour was lower than its corresponding sun-dried sample. Hesam *et al*. (2012) had also reported significant difference among the EC_{50} values recorded for three potato extracts.

3.3. Total Phenolic Content (TPC) of Fresh Tubers and Flour Samples

Phenolic compounds are secondary plant metabolites present in fruits and vegetables which can contribute directly to antioxidant properties as a result of their redox properties (Zheng and Wang, 2001; Brown, 2005). Drying processes caused a dramatic increase in the TPC of the flour samples and the values obtained ranged from 4.51–9.82 mg GAE/g for fresh samples and 18.72–21.60 mg GAE/g for flour samples (Figure 2). Ruttarattanamongkol *et al*. (2015), Bellail *et al*. (2012) and Tokusoglu and Yildirim (2012) had reported similar observations in their studies. The increment in the TPC of the samples might have been caused by the degradation of cellular constituents during heat treatment and resulted into the breakdown and release of some free phenolics. However, the TPC of the flour samples varied significantly according to drying methods and the effect was observed to be tuber dependent. Different total phenolic contents according to drying methods had also been reported for sweet potato flour (Ahmed *et al*., 2010). The TPC of flours obtained in this study was comparable to the values of 15–34 mg GAE/g for hot air dried sweet potato flours, 32.2 mg GAE/g DW for a red fleshed sweet potato and 9.60–54.30 mg/g DW reported by Ruttarattanamongkol *et al*. (2015), Cevallos-Casals and Cisneros-Zevallos (2003) and Ji *et al*. (2015) respectively. On the other hand, many other researchers had recorded relatively lower values for the TPC of potato and sweet potato flours (Al-Saikhan *et al*., 1995; Ahmed *et al*., 2010; Hesam *et al*., 2011; Koala *et al*., 2013). This could be due to the variation in genetic composition, sample treatment, processing method, extraction condition and environmental factors such as abiotic stress, nutrients, weather, water supply, temperature and other growing conditions (Vinson *et al*., 1998; Kaur and Kapoor, 2002; Reyes and Cisneros-Zevallos, 2003; Huang *et al*., 2005; Mohagheghi Samarin *et al*., 2008; Hamouz *et al*., 2007).

3.4. Beta-carotene Content (BCC) of the Flour Samples

Beta carotene is an important precursor of vitamin A found in plants and it ranged from 1.88 µg/g in the sun-dried WFSP and solar-dried IP flours to 3.81 µg/g in the sun-dried and solar-dried OFSP flours (Table 3). OFSP is known to be a rich source of β-carotene (Amajor *et al*., 2011). Ezekiel *et al*. (2013) had also stated that potato cultivars with yellow or orange flesh contained more carotenoids compared to cultivars with white flesh. However, the beta-carotene content (3.81 µg/g) recorded for sun and solar dried OFSP flour samples indicated that the two drying methods had similar effect on the beta-carotene of OFSP. It was observed that the beta-carotene content of IP varied significantly ($p<0.05$) with the drying methods. Beta-carotene content of 0.42–2.19 DW µg/g in sixteen native Andean potato varieties, 0.181–0.254 mg/100g in two sweet potato varieties and 0.18–226 µg/g FW in sweet potato cultivars had been reported by Andre *et al*. (2007), Tumuhimbise *et al*. (2013) and Teow *et al*. (2007) respectively. Amajor *et al*. (2011) reported a lower beta carotene content of 0.02×10^3–3.99×10^3 µg/100g carotenoids in five varieties of sweet potato. High beta carotene values as compared to those recorded in this study had been reported by many other researchers (Haile *et al*., 2015; Nicanuru *et al*., 2015; Ruttarattanamongkol *et al*., 2015; Nkom and Ojimelukwe, 2016). Variation in beta-carotene content may be due to differences in varieties, cultivars, growing conditions, stages of maturity, harvesting and post-harvest handling, processing and storage, air and soil temperature, radiation, location, fertilization, soil moisture and other environmental factors (K'Osambo *et al*., 1998; Rodriguez-Amaya, 2000; Mbwaga *et al*., 2007; Ukom *et al*., 2009). Beta carotene may be one of the major phytochemicals contributing to the total antioxidant activities of the flour samples studied.

3.5. Total Flavonoids Content (TFC) of the Fresh and Flour Samples

Drying generally increased the TFC of Irish potato, white-fleshed and orange-fleshed sweet potato flour samples (Figure 3). The TFC ranged from 0.22 mg QE/g in IP to 0.36 mg QE/g in OFSP for the fresh samples and 0.50 mg QE/g in solar-dried OFSP to 1.15 mg QE/g in sun-dried OFSP for the flour samples. Comparable value of 50.77 mg QE/100g on fresh weight basis in sweet potato variety and significantly high range of value (96.91–579.63 mg QE/g) in sweet potato varieties had been reported by Eleazu and Ironua (2013) and Salawu *et al*. (2015) respectively. Drying methods had significant effect ($p<0.05$) on the TFC of the sweet potato varieties (OFSP and WFSP) flour samples with the highest values recorded for the sun-dried samples. The low value of TPC recorded for the solar-dried OFSP flour may be an indication that flavonoid is not one of the major bioactive compounds responsible for the total antioxidant activities of potato and sweet potato flours investigated. Flavonoids are polyphenolic compounds which had been attributed to free radical scavenging activity in foods and inhibition of proliferation of a wide variety of cancer cell lines through scavenging or chelating process (Cook and Samman, 1996; Huang *et al*., 1999; Kessler *et al*., 2003; Pourmorad *et al*., 2006).

Table 1. Flour yields of OFSP, WFSP and IP samples from different drying methods

Samples	**Drying Time (Days)**	**Fresh weight (kg)**	**Flour yield (%)**
$OFSP_{Sun\text{-}dried}$	3	5.00	18.00^{b}
$OFSP_{Solar\text{-}dried}$	5	5.00	17.20^{c}
$WFSP_{Sun\text{-}dried}$	3	5.00	19.00^{a}
$WFSP_{Solar\text{-}dried}$	5	5.00	15.60^{e}
IP $_{Sun\text{-}dried}$	3	5.00	15.20^{f}
IP $_{Solar\text{-}dried}$	5	5.00	16.00^{d}

Values with different superscript differ significantly ($p<0.05$) from each other. **OFSP**= Orange fleshed sweet potato, **WFSP**= white fleshed sweet potato, **IP**= Irish potato.

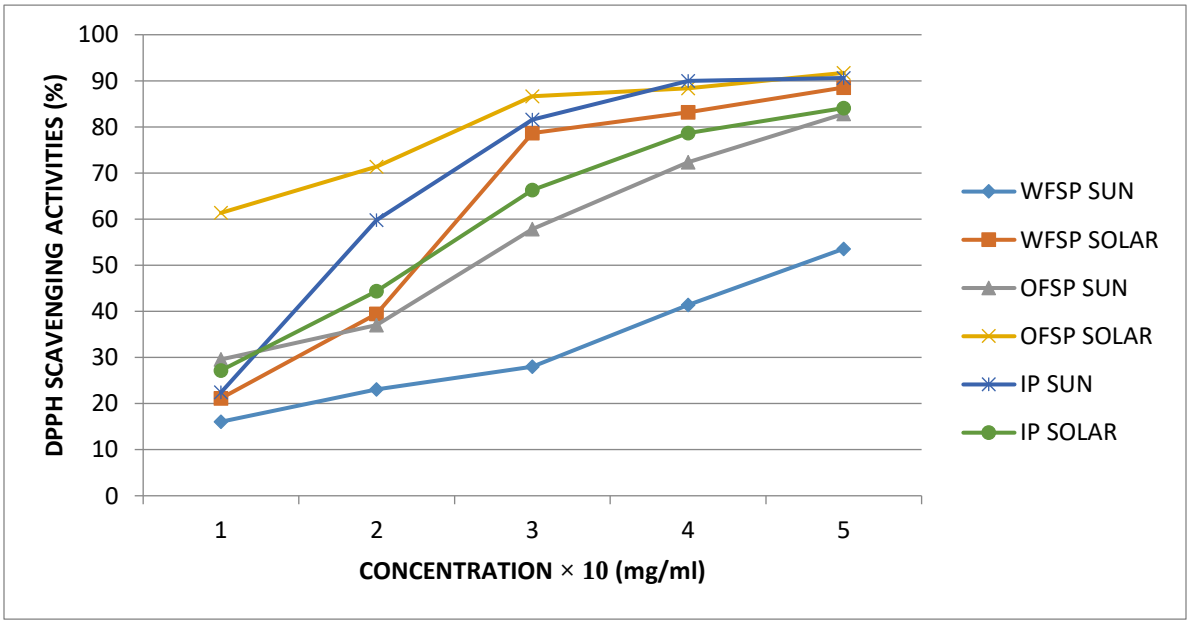

Figure 1. DPPH radical scavenging activities of OFSP, WFSP and IP flour samples as affected by drying methods

Table 2. Effect of drying methods on the total antioxidant activities of WFSP, OFSP and IP flour samples

Samples	(%) Inhibition at 50 mg/ml	EC_{50} value (mg/ml)
$WFSP_{Sun\ dried}$	53.58±1.33[c]	45.214[a]
$WFSP_{Solar\ dried}$	90.62±3.26[a]	22.853[c]
$OFSP_{Sun\ dried}$	84.10±2.45[b]	26.429[b]
$OFSP_{Solar\ dried}$	90.62±1.97[a]	0.584[e]
$IP_{Sun\ dried}$	90.62±2.98[a]	17.143[d]
$IP_{Solar\ dried}$	84.10±2.21[b]	22.857[c]

Each value is a mean of three determinations ± standard deviation. Means within column not followed by the same superscripts are significantly different (*p<0.05)* from each other. **OFSP**= Orange-fleshed sweet potato, **WFSP**= white-fleshed sweet potato, **IP**= Irish potato.

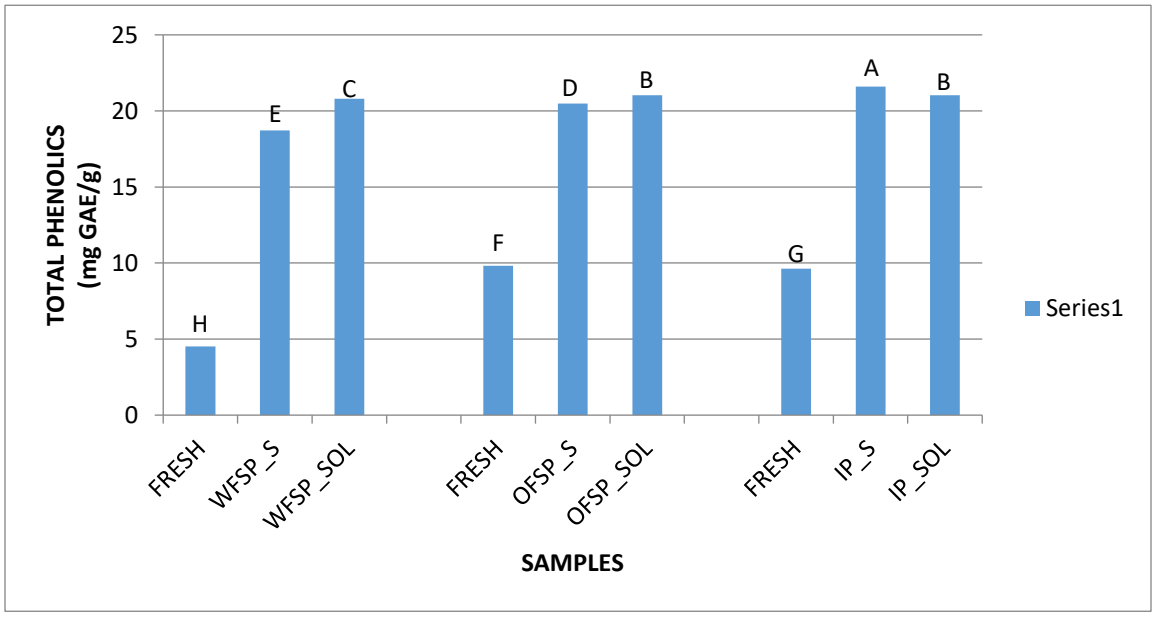

Figure 2: Total Phenolics Content (TPC) of fresh and flour samples. Bar charts not followed by the same alphabets are significantly different (*p<0.05)* from each other. S= Sun-dried, SOL= Solar-dried.

Table 3: Beta-carotene of OFSP, WFSP and IP flours as affected by drying methods

Samples	Beta-Carotene Content (TCC) (µg/g)
WFSP $_{\text{Sun dried}}$	1.88±0.01^{c}
WFSP $_{\text{Solar dried}}$	1.90±0.02^{bc}
OFSP $_{\text{Sun dried}}$	3.81±0.01^{a}
OFSP $_{\text{Solar dried}}$	3.81±0.02^{a}
IP $_{\text{Sun dried}}$	1.92±0.03^{b}
IP $_{\text{Solar dried}}$	1.88±0.01^{c}

Values are means of three determinations ± standard deviations. Means not followed by the same superscripts are significantly different (*p<0.05).* **OFSP**= Orange-fleshed sweet potato, **WFSP**= white-fleshed sweet potato, **IP**= Irish potato.

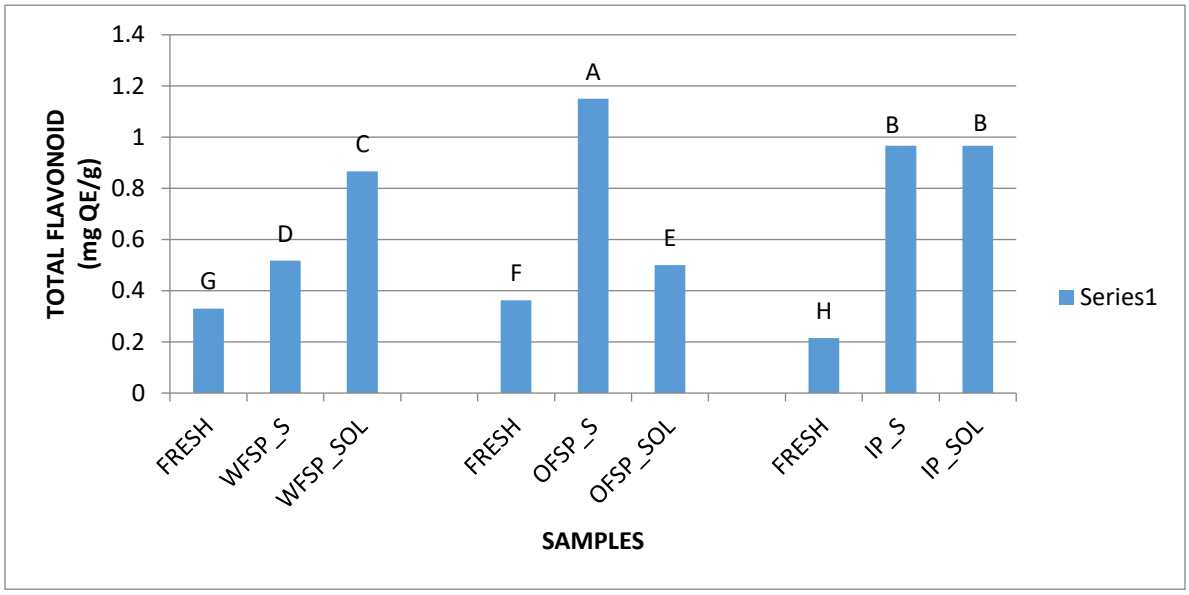

Figure 3: Total Flavonoids Content (TFC) of fresh and flour samples. Bar charts not followed by the same alphabets are significantly different (*p<0.05)* from each other. S= Sun-dried, SOL= Solar-dried.

4. Conclusions

Flours were produced from IP, OFSP and WFSP using two drying methods (sun and solar). The effect of drying methods on the flour yields was observed to be significant (p<0.05) irrespective of the tuber and varietal differences. The DPPH free radical scavenging activities of the flours were noticed to be drying methods and extracts' concentrations dependent. It was shown that drying processes increased the TPC and TFC of the flour samples. The variation in the effects of drying methods on the total antioxidant activities, TPC, beta-carotene content and TFC were observed to be tuber (Irish potato and sweet potato) dependent. Sun-drying method had significant less favorable effect on the

WFSP sample compared to solar-drying method. Although solar-dried OFSP flour had the highest total antioxidant activity, sun-drying method produced IP and OFSP flours which had the highest TPC and TFC respectively. Based on this observation, it may be suggested that the total phenolics and the beta-carotene are majorly responsible for the antioxidant activities of the flour samples investigated. This study had shown that IP, WFSP and OFSP flours are good sources of phytochemicals most of which are responsible for their antioxidant activities as well as vitamin A supplementation in the body. Selection of potato and sweet potato varieties with high levels of phytonutrients should go hand in hand with the adoption of technological techniques with high favorable effect on the yield, antioxidant capacity and phytochemical composition of the plants in order to enhance output and maximize health benefits derivation.

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EFFECTS OF METHYL JASMONATE FUMIGATION AND PACKAGING ON CHILLING INJURY AND PHYSIOCHEMICAL QUALITY CHANGES OF STORED GREEN BELL PEPPERS

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ABSTRACT

Bell peppers are widely grown fruit with high nutritional and commercial value. However, prolonged low-temperature storage subjects bell peppers to a high risk of chilling injuries (CI). In this study, combination treatments with various concentrations of methyl jasmonate (0, 10, 20 & 30 mmol/L MeJA) and packing in oxygen transmission rate polyethylene bags (OTR-PE 5,000-6,000 $cm^3/m^2/d$) were explored to minimize the CI incidence in bell peppers under prolonged low temperature storage (4 °C and 90% RH). Untreated bell peppers (no MeJA treatment, no OTR-PE bag) were the control group, and bell peppers stored in OTR-PE bags without MeJA fumigation were included in comparisons. Sampling and other observation were done every 5 days over 30 days in total. In general, the 30 mmol/L MeJA treated bell peppers stored in OTR-PE bags were well controlled against CI induced changes in headspace gas, respiration rate, ethylene, color (L*, H° & C*), chlorophyll content, CI index, electrolytic leakage, weight loss, and firmness. Also, the lower MeJA concentrations (10 and 20 mmol/L MeJA) provided significant control of CI in bell peppers. These treatments retained high levels of ascorbic acid and free polyamines in the bell peppers. Packing in OTR-PE bags reduced the CI incidence compared to the control group. Overall, the MeJA treatments retained the commercial value of bell pepper fruit better (>80%) than the OTR-PE bags (33%) or the untreated control (23%) over 30 days of storage.

1. Introduction

Chilling injury (CI) is a postharvest physiological disorder, deleterious to numerous horticultural products grown in tropical and subtropical regions. The CI develops during extended storage below an experimentally determined threshold temperature that is specific to the particular product (Wang, 1989). Numerous physiological and biochemical changes occur in chilling sensitive produce during prolonged refrigerated storage, leading to a variety of CI symptoms such as pitting, cell membrane degradation, discoloration, immaturity, wilting, off-flavor and decay (Venkatachalam and Meenune, 2015; Wang, 2010). Green bell pepper (*Capsicum annum* L.) is an essential nutritious and decorative plant used in numerous cuisines, but it suffers detrimental changes during storage under too high or too low temperatures (Fung et al., 2004). Especially during prolonged storage at a

refrigerated temperature (4-7 °C), the bell peppers are extremely susceptible to CI symptoms including pitting, seed browning, tissue discoloration, wilting and scalding. There are numerous studies on the control of CI incidence or providing resistance against the chilling stresses by use of packaging, intermittent warming, coating or fumigation with plant growth regulators (Cao et al., 2012; Hassan et al., 2014).

Methyl jasmonate (MeJA) is a naturally occurring plant growth regulator that endogenously signals the defense system of the plant to protect it against abiotic and biotic stresses. The use of MeJA is widely spread due to its beneficial effects on controlling postharvest losses, especially CI disorders of numerous plant products (Dar et al., 2015; Gonzalez-Aguilar et al., 2003; Jin et al., 2009; Perez et al., 1993). In the recent years, the application of MeJA has increased because it is low cost, easy to apply, colorless, flavorless and the safest among the various effective postharvest treatments. MeJA can be applied to plants by fumigation and/or by dipping, and many studies have found that fumigation is the most efficient of these two techniques, partly due to the volatile nature of MeJA (Venkatachalam and Meenune, 2015). However, several studies have reported that the modes of action of MeJA on controlling CI in plants are unclear, although prior studies have shown that MeJA treatment induces several biosynthetic enzymes and/or metabolites such as antioxidant enzymes, prolines, and polyamines produced by ornithine decarboxylase and ornithine aminotransferase enzymes in chilling-sensitive plants. These effects are likely significant to the control of CI incidence in plants (Jin et al., 2009; Perez et al., 1993; Zhang et al., 2012).

The use of combined post-harvest treatments has grown vastly due to their efficacy in the control of postharvest losses of fresh produce. Modified atmospheric packaging (MAP) is inexpensive and also a widely used method to control CI and fungal decay in several crops (Beaudry, 2000; Gonzalez-Aguilar et al., 2004a; Singh et al., 2014). With a MAP, it is possible to deplete the O_2 level and increase the CO_2 level in the package, which alters the rates of biochemical degradation in fresh produce and consequently extends its shelf life. When green bell peppers were stored at a low temperature (5 °C) in passive MAP packaging, this increased their shelf life up to 21 days (Gonzalez-Aguilar et al., 2004a). Fresh cut bell peppers stored at 0 °C under the active MAP in a high-density polyethylene film had no quality losses for up to 10 days (Manolopoulou et al., 2012). Buta and Moline (1998) found that MeJA treatment of fresh cut bell peppers controls microbial contamination for two weeks under storage at 10 °C. MeJA treated sweet peppers had increased alternative oxidase (AOX) transcript levels, along with the reduced level of CI incidence (Fung et al., 2004). While numerous studies address controlling the CI incidence of bell peppers, they tend to focus on an individual postharvest technique applied to fresh-cut produce. In this study, we investigated the combination of postharvest treatments by MeJA and passive MAP, to control the CI incidence in whole green bell pepper fruit under refrigerated storage.

2. Materials and methods

2.1. Plant materials and MeJA treatments

Mature green bell peppers (*Capsicum annuum var. annuum*) with near similar sizes were harvested, avoiding any apparent damage, from a commercial orchard in southern Thailand. The harvested fruits were transported to the laboratory within 4 h. They were washed with filtered tap water, and their surfaces were thoroughly dried in ambient air by an electric fan. After this, they were treated without delay. For the MeJA treatments, the bell pepper fruit was divided into four treatment groups, treated at concentrations of 0 (control), 10, 20 and 30 mmol/L. Each group was dealt with separately in an air sealed container (20 L) for 24 h at ambient temperature (~27 ºC). After the fumigation treatment, the fruit samples were

ventilated for at least 4 h, then packaged and stored.

2.2. Packaging and Storage

For each replication of a treatment group, three bell peppers were placed in an oxygen transmission rate polyethylene (OTR-PE) bag rated at 5,000-6,000 $cm^3/m^2/d$ and water vapor transmission rate (WVTR) of at 27.6. The OTR-PE bags were 8x12 inches size with 25 µM thickness, and they were acquired from Thantawan Industry Public Company Limited, Thailand. The untreated fruits (0 mmol/L MeJA) were divided into two groups: without a package (*Control*) and with a package (*OTR-PE*). The MeJA treated bell peppers stored in OTR-PE packages were labeled by their MEJA treatment. The OTR-PE bags were sealed with an impulse sealer (Hand impulse sealer, model PCS 300C). After that, all the treated and untreated samples were stored at 4 °C with 90% RH for up to 30 days. The shelf-life assessment was discontinued for a package when there was visible microbial growth and/or severe chilling injury symptoms. At 5-day intervals, the bell peppers were removed from refrigerated storage and were allowed to reach the ambient temperature (≃4 hrs), and then various observations were made, described next.

2.3. In-package gas composition (O_2 & CO_2), respiration rate and ethylene production

Before opening the packages, the headspace gas compositions were measured. A 6 mL gas sample was withdrawn from the package and injected into a gas chromatograph (GC) equipped with Porapack N column and a thermal conductivity detector (TCD). External gas standards were used to identify and quantify the package headspace gasses, and the results are expressed as percentages (%) by volume or equivalently by molar amounts. To determine the respiration rate, three bell peppers were enclosed in a 2 L glass jar for 2 h at 20°C, and then a 1 mL headspace sample was withdrawn from the jar and injected into the GC. The results are expressed as mg CO_2 kg^{-1} h^{-1}. For ethylene production, a 1 mL gas sample from the jar was injected into a GC equipped with a flame ionization detector, set at column temperature 140 °C with an 80/100-mesh Porapack Q column. The results are expressed as nL g^{-1} h^{-1}.

2.4. Weight loss

The initial and final physiological weights of bell peppers in each treatment group were measured using an electronic weighing balance. The weight loss was calculated as follows:

$$W_L\,(\%) = [(W_I - W_F)/ W_I] \times 100$$

where $\mathbf{W_L}$ is the weight loss (%), $\mathbf{W_I}$ is the initial weight (g), and $\mathbf{W_F}$ is the final weight of the bell peppers.

2.5. Surface color

Four sides of each bell pepper were measured for color (CIE-L*, a* and b*) using a Hunter colorimeter calibrated with a white and a black tile. The values of a* and b* were converted into hue ($\tan^{-1}$ b*/a*) and chroma $[(a^{*2} + b^{*2})^{1/2}]$ as described by Francis (1980).

2.6. Firmness

The firmness of the bell peppers was analyzed by measuring the maximal compression force using a Texture Analyzer (TA-XT2i, Stable Micro System, UK) equipped with a stainless steel compression plate and using a 50 N load cell. The results are expressed as kgf.

2.7. Chilling injury (CI) index and marketability

The CI index was measured following the method of Liu et al. (2013). The CI index is based on the severities of calyx browning, surface pitting, texture softening, shrinkage and decay, and was determined at each observation time point. The determination relies on human sensory perceptions and can be considered subjective and semi-quantitative. Ten trained panelist were used to evaluate the CI index for the bell pepper samples during the experiment. The panelists scored characteristics on a five-

point scale with 0= none; 1= slight (CI is below 25%); 2= moderate (CI is between 25% and 50%); 3= moderate-severe (CI is between 50% and 75%); 4= severe (CI is above 75%). The CI index was calculated as follows:

$$CI\ index = \frac{SUM\ [(CI\ level)\ x\ (number\ of\ bell\ peppers\ at\ the\ CI)]\ level)]}{(Total\ number\ of\ bell\ peppers\ in\ the\ treatment)}$$

The count of bell peppers completely without detectable decay (CI index 0) at the end of storage experiment is reported as the fraction of the total, by treatment, and this is here called marketability. The CI and marketability are both expressed as percentages.

2.8. Electrolytic leakage

Electrolytic leakage was measured in accordance with the method of Saltveit (2002). A stainless steel borer was used to cut bell pepper tissue discs about 5 mm in diameter. Nine discs from one fruit were incubated in 20 mL of 0.2 M mannitol solution. Conductivity at the beginning of incubation (EL_0) was measured using a conductivity meter, and the disc samples were then incubated on a shaker for 24 h, at 25 °C and 60 x g. The conductivity of the solution was measured again after the 24 h incubation (EL_{24}). The solutions with bell pepper discs were frozen at -20 °C for 24 h, then thoroughly thawed, and finally measured for their total conductivity (EL_t). The electrolytic leakage in bell peppers was calculated using the following formula, and the results are expressed as percentages.

$$EL\ (\%) = [(EL_{24} - EL_0) / (EL_t - EL_0)] \times 100$$

2.9. Total chlorophyll

Total chlorophyll contents of the bell peppers were measured following the method of Xing et al. (2011) with slight modifications. A bell pepper tissue sample (10 g) was extracted in 80% acetone, and the mixture was centrifuged at 6000 x g, at 4 °C for 10 min. The supernatant was collected and measured for absorbance at the wavelengths 645 and 663 nm. The following equation was used to quantify the chlorophyll contents of bell peppers.

$$Chlorophyll\ content\ (mg/g) = 17.76 \times A_{646.6}\ nm + 7.34 \times A_{663.6}\ nm$$

2.10. Ascorbic acid content

Ascorbic acid contents of the bell peppers were determined using the 2, 6,-dichloro-phenol-indophenol titration method as described by Jones and Hughes (1983). A bell pepper tissue sample (10 g) was homogenized in 10 mL of 3% metaphosphoric acid; the homogenate was made up to 100 mL volume and centrifuged at 3000 x g for 15 min at 25 °C. Ten milliliters of the supernatant was then titrated against 2, 6-dichloroindophenol, which had already been standardized against standard ascorbic acid, and the results are expressed in mg g^{-1}.

2.11. Polyamine analysis

The free polyamine levels in the bell peppers were quantified following the method of Cao et al. (2012) with slight modifications. Bell pepper tissue (5 g) was homogenized with a chilled mortar and pestle, with 3 mL of 0.4 M $HClO_4$, and 1 mL of 0.8 mM 1, 6-hexane-diamine as an internal standard. Then the homogenate was centrifuged at 4 °C for 20 min at 14,000 x g for 15 min. The collected supernatant was dansylated and extracted with 500 µL of toluene. The organic phase was then dried under a stream of N_2 at 70 °C, and then suspended in 200 mL of acetonitrile (HPLC grade) and filtered using a 0.2 µm pore filter before HPLC analysis. Polyamines were eluted through a 200 mm x 4.6 mm reverse-phase C18 column packed with 5 µm Hypersil ODS resin. Elution was performed at a flow rate of 1.5 mL min^{-1} with a gradient of 60-90% acetonitrile over 25 min at 35 °C. The dansylated polyamines in the extracts were detected by fluorescence at the emission wavelength of 447 nm and quantified from peak areas using 1, 6-hexanediamine as the internal standard, and using the standard curves for Put, Spd, and Spm.

2.12. Statistical analysis

All the experimental data were conducted using SPSS software for Windows (SPSS Inc., Chicago, IL). The mean values were calculated and reported as the mean ± SD (n = 6). The data were plotted using SigmaPlot. The experiments were conducted in a factorial design, subjected to analysis of variance (ANOVA) and significant differences between the treatments were decided by *Fisher's* least significant difference test at a confidence level of $P<0.05$.

3. Results and discussions

3.1. Respiration and Ethylene production

The changes in O_2 and CO_2 contents in package headspace gas of OTR-PE bags with bell peppers in low temperature storage are shown in Fig. 1A-B. The trend in O_2 content was increasing while the CO_2 content decreased, consistently throughout the study period regardless of treatment. The untreated bell peppers caused more substantial changes to the package atmospheric gas than the MeJA treated bell peppers ($P<0.05$). Effects of the MeJA treatments were dose-dependent in a consistent manner, showing significant control of chilling induced respirational changes in the bell peppers stored in OTR-PE bags, especially at the highest 30 mmol/L MeJA dose level. Several studies have observed similar trends of declining O_2 and rising CO_2 levels when bell peppers are stored under MAP (Manolopoulou et al., 2012; Singh et al., 2014). A steady state of the headspace gas in the bag is achieved when the uptake of O_2 and production of CO_2 by the bell peppers are balanced by the gas fluxes permeating through the packaging film, assuming constant respiration rate. Beaudry (2000) reported that when fresh produce is stored in bags at a low temperature, a solid composition of the headspace gas will not be achieved rapidly. It should be considered that the higher content of O_2 and the lower content of CO_2 inside the MAP packages containing the MeJA treated peppers is due to the effect of MeJA on reducing respiration rate as can be observed in Fig. 1C. Li et al. (2011) found that 1-MCP treatment had changed the headspace gasses in MAP bag, in the case of stored bell peppers. Furthermore, Gonzalez-Aguilar (2003) observed that papaya fruit treated with MeJA and stored under passive MAP conditions maintained a steady oxygen level in the headspace gas, contrary to the untreated fruit.

The respiration rates for all experimental cases are shown in Fig. 1C; the cases were untreated bell peppers were stored with and without OTR-PE bags, and MeJA treated ones in bags. The results indicate that the respiration rate of bell peppers steadily decreased throughout the storage ($P<0.05$), across all treatments. The control bell peppers stored without MeJA treatment or MAP packaging retained a higher respiration rate than the cases with actual treatment. Li et al. (2011) reported that MAP storage reduces the respiration rate of bell peppers by controlling metabolic activity and ethylene sensitivity, due to effects of the package on headspace gas composition during storage. This agrees with the present result that the control samples had the highest respiration rates. Rahman et al. (1993) observed that bell peppers stored under low oxygen conditions could have reduced respiration rates. Lim et al. (2007) reported that bell peppers stored at 7 °C showed higher respiration rates and increased susceptibility to chilling injuries relative to those stored at 10 °C. In the current study, the MeJA concentrations 20 and 30 mmol/L gave mutually similar and significantly lower respiration profiles than the lowest concentration MeJA treatment ($P<0.05$), demonstrating a dose-dependent response that saturated. Gonzalez-Aguilar (2003) observed no significant differences between treatments with different MeJA concentrations, on controlling the respiration rate of papaya fruit, which might indicate similar saturation at the and the ethylene production (D), for bell peppers stored at 4°C and 90% RH.

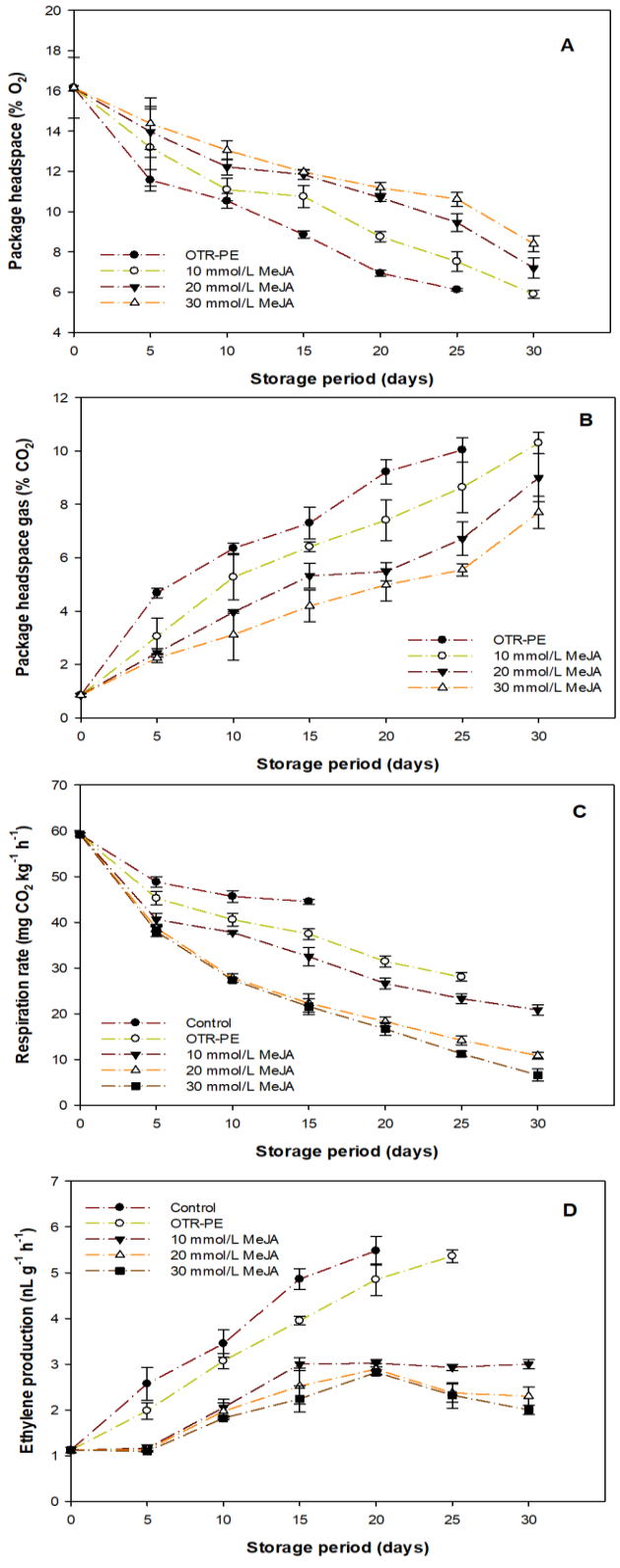

Fig. 1. Time profiles of the package headspace gas composition (A-B), the respiration rate (C) and the ethylene production (D), for bell peppers stored at 4°C and 90% RH.

Production of ethylene by treated and untreated bell peppers in refrigerated storage is shown in Fig. 1D. The ethylene production initially increased steadily across all treatments, but the MeJA treatments gave steady or decreasing ethylene levels from about day 20 of storage onwards. The MeJA treatments again show consistent dose-dependent responses, although with saturation, so the two highest doses gave similar responses. The MeJA treatments significantly reduced ethylene production by the bell peppers, in comparison to the other treatments ($P<0.05$). Generally, bell pepper is categorized as a non-climacteric fruit, which naturally produces minute amounts of ethylene (Hassan et al., 2014; Lim et al., 2007). However, several studies have reported increased ethylene production as an indicator of chilling injury in bell peppers (Sanchez-Bel et al., 2012; Wang, 1989; Wang et al., 2012). In addition, a lower O_2 level is directly related to the onset of off-flavor formation and accumulation of ethylene and acetaldehyde (Beaudry et al., 2000). The suppressive effects of MeJA on ethylene production in bell peppers could be achieved by reducing the enzymatic activities involved in the biosynthesis of ethylene in plants. Soto et al. (2012) reported that exogenous treatment by MeJA decreased aminocycloprepane-1-carboxylate oxidase (ACO) and 1-aminocyclopropane-1-carboxylate synthase (AOS) in peach fruit, and subsequently the fruit produced less ethylene.

3.2. Chilling injury incidence

3.2.1. Surface color and chlorophyll content

The color parameters (L*, H° & C*), decreased consistently over time for the fruit surface, regardless of the treatment of bell peppers, including both actual treatments and untreated control (Fig. 2A-C). The bell peppers in the control group and those with OTR-PE bags had a more severe decline of the surface color parameters than those with MeJA treatments. In comparison to controls, the bell peppers in OTR-PE bags held their surface color slightly better ($P<0.05$). The MeJA treatments gave significantly different time

profiles of surface color when compared to controls ($P<0.05$), and while the plots subjectively indicate a consistent dose-dependent response, the differences between the MeJA dose levels were not statistically significant. Commonly, the surface color changes of bell peppers are directly influenced by the total chlorophyll content, as this is the predominant pigment in green bell peppers, and various environmental disturbances tend to reduce the chlorophyll level (Ilic et al., 2009; Wang et al., 2012). Lim et al. (2007) observed that chilling induced reduction of the chlorophyll content affected the bell peppers' surface L*, H°, and C* values. The chlorophyll contents of stored bell peppers in the current study are shown in Fig. 2D, corroborating the connection to surface color.

Throughout the study period, the chlorophyll content was lower in the untreated bell peppers (control) than in the treated peppers. The OTR-PE bags suppressed the loss of chlorophyll from the bell peppers, and there were no significant differences between any of the actual treatments ($P\geq0.05$). Ethylene is a vital phytohormone that plays numerous roles in altering the biophysiology of plants, especially in degrading plant chlorophyll content. Purvis and Barmore (1981) reported that the degradation of chlorophyll in the peel of citrus fruit was accelerated by ethylene. Furthermore, chlorophyllase is the key enzyme involved in the degradation and catalyzes the hydrolysis of ester bonds in chlorophyll, which forms chlorophyllide and phytol (Tsuchiya et al., 1999). Several studies report that plants under prolonged refrigerated storage tend to have high chlorophyllide activities (Hershkovitz et al., 2005; Jemriic et al., 2004; Liu et al., 2013). Generally, the mode of action of MeJA varies by the plant crop, and MeJA treatment of apple and guava fruit decreased their chlorophyll contents (Gonzalez-Aguilar et al., 2004b; Perez et al., 1993). Overall, these prior findings corroborate the chlorophyll observations in the present study as reasonable.

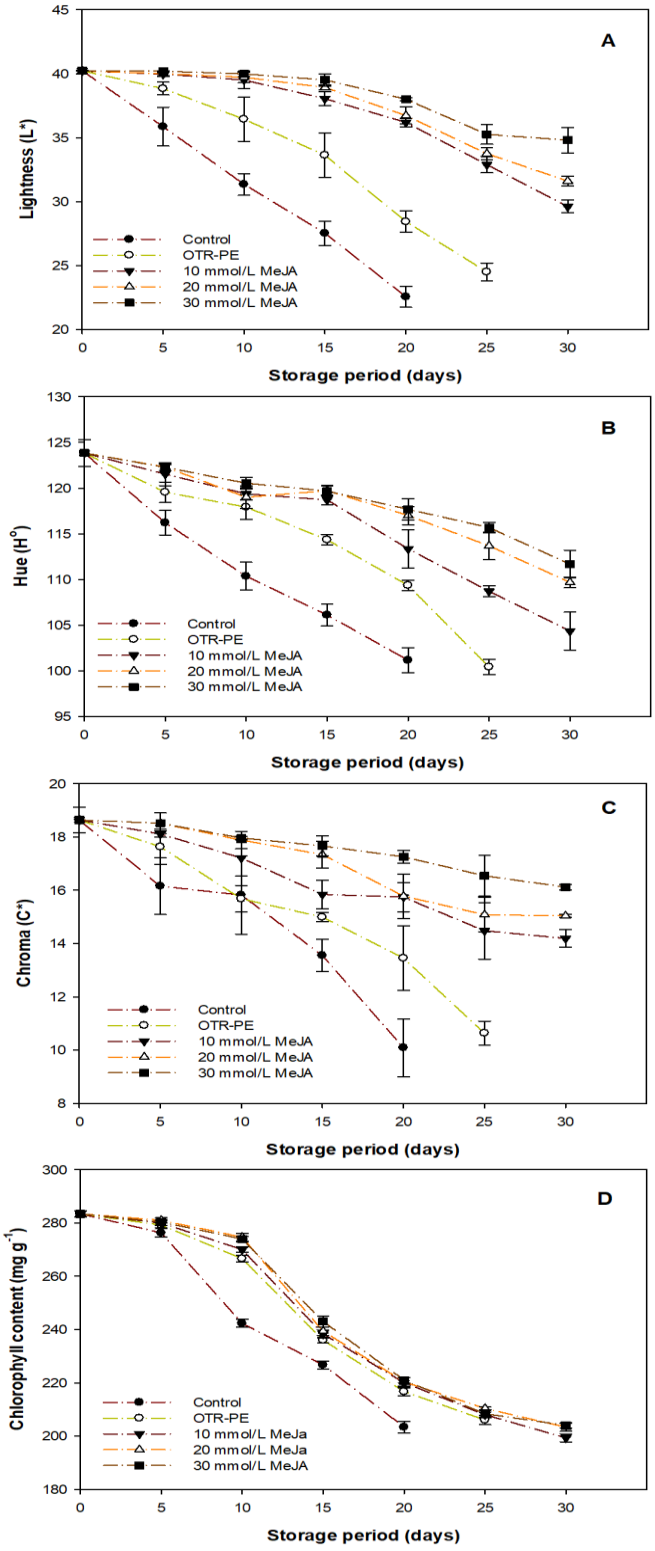

Fig. 2. Time profiles of the surface color parameters lightness (A), hue (B), and chroma (C), and the chlorophyll content (D), for bell peppers stored at 4°C and 90% RH.

3.2.2. CI index and marketability

The time traces of chilling injury (CI) index are shown in Fig. 3A for bell peppers with the various treatments. The CI index had an increasing time trend across all the treatments, as might be expected. The control group had the highest CI index, followed by OTR-PE and MeJA treatments consistently. The OTR-PE bag acted as a barrier and lowered the CI index in the bell peppers, and the response to MeJA appears again dose-dependent but saturating, so the two highest doses gave very similar responses. The MeJA treatments reduced the severity of chilling injury symptoms statistically significantly. The electrolytic leakage (EL) increased consistently throughout the storage (Fig. 3B). Initially, the EL showed no differences between the various treatments, but with prolonged storage, the MeJA treatments were the best in controlling the EL of bell peppers while also the OTR-PE bags significantly protected the bell peppers against chilling induced EL. Lim et al. (2007) reported that green bell peppers were more susceptible to chilling injuries when matured, and these had plenty of electrolyte leakage from the skin during prolonged low-temperature storage. Normally, chilling injury induces membrane-degrading enzymes in bell peppers, and this increases the membrane permeability so that EL follows (Xing et al., 2011). The induced EL after prolonged exposure and chilling injuries is due to the breakdown of membrane lipids (Liu et al., 2013). Several studies have found that MeJA treatment reduces the membrane degradation in various products and controls the membrane damage, as well as EL leakage (Jin et al., 2009; Meng et al., 2009; Venkatachalam and Meenune, 2015). The marketabilities of bell peppers with the various treatments are shown in Fig. 4. Marketability tended to decrease especially towards the end of the study period. However, the MeJA treated peppers retained the highest percentage of their commercial value (>80%) among the various treatments. This response was dose-dependent, and the highest MeJA dose gave 93% marketability at 30 days of storage, being significantly better than the others. Without MeJA treatment even with OTR-PE bags only a small <35% fraction of the bell peppers was marketable at 30 days of storage. This was mainly due to severe microbial growth (and induced decay) on the bell pepper surfaces. The barrier effect of OTR-PE bags was still significant, and the untreated control group had the poorest marketability.

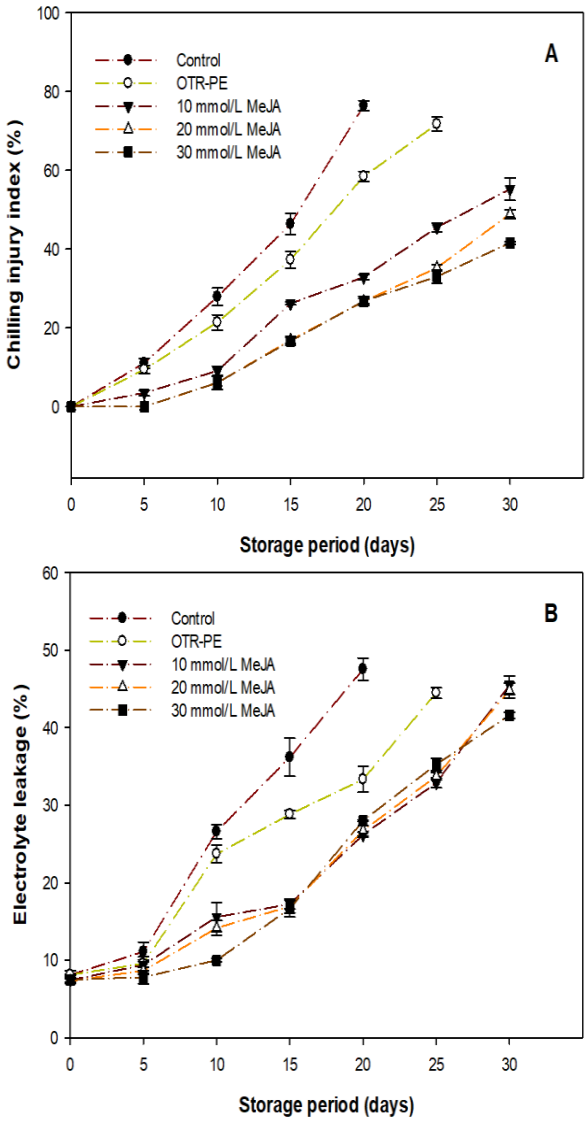

Fig. 3. Time profiles of chilling injury index (A) and electrolytic leakage (B), for bell peppers stored at 4°C and 90% RH.

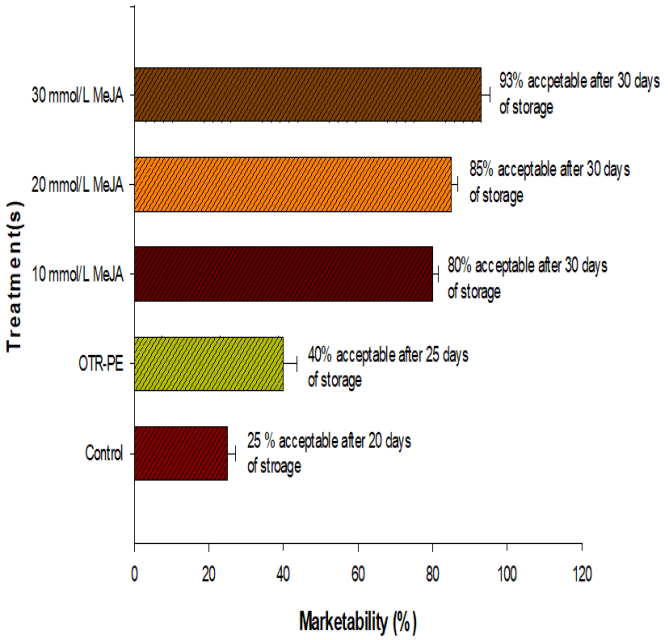

Fig. 4. The marketability of bell peppers stored at 4°C and 90% RH was dramatically affected by choice of treatment.

3.2.3. Weight loss and Firmness

The changes in physiological weight are shown in Fig. 5A. During storage, the weight loss increased consistently with time across all the treatments, and the MeJA treatments were significantly (*P<0.05) better than the others in controlling the loss. The use of OTR-PE bags was still a significantly better option than no actual treatment (control). Wang (2010) reported that film packaging, and/or other external treatments that reduce the water loss, alleviate chilling injuries in plant products. Comparison of the MeJA treatments showed no significant dose dependence in the control of bell pepper weight loss. At the end of storage, the MeJA treated bell peppers had lost about 5% of weight while treatments without MeJA gave losses exceeding 10%. The weight loss in bell peppers was likely affected by CI associated cellular breakdown, loss of membrane integrity, and the apparent removal of epicuticular waxes (Gonzalez-Aguilar et al., 2000). The time profiles in Fig. 5B shows the consistent loss of firmness for each treatment group of bell peppers. The MeJA treatments gave the best retention of firmness in a dose-dependent manner, with the highest dose level preventing immediate initial losses (up to 10 days of storage) that occurred with the other treatments. Tsegay et al. (2013) reported progressively increasing the loss of firmness in bell peppers with storage time and attributed this to the activity of cell wall degrading enzymes. Several studies have found that MeJA treatment of plant products reduces the activities of cell degrading enzymes during prolonged storage at refrigerated temperatures (Jin et al., 2009; Venkatachalam and Meenune, 2015).

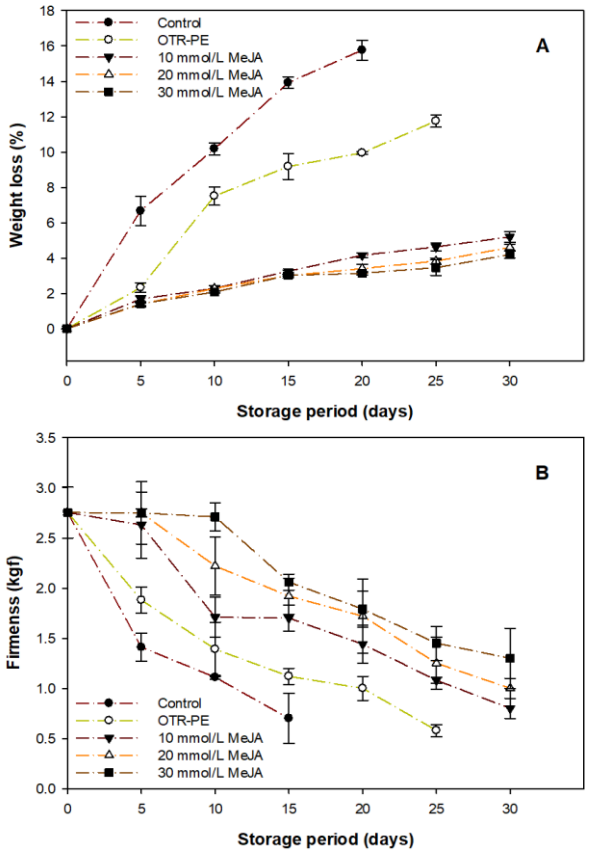

Fig. 5. Time profiles of weight loss (A) and firmness (B), for bell peppers stored at 4°C and 90% RH.

3.2.4. Ascorbic acid and Polyamine content

The time profiles of ascorbic acid (AsA) content are shown in Fig. 6A. for the variously treated bell peppers. The overall trend in AsA

content was decreasing regardless of treatment, and only the MeJA treatments prevented initial decline up to 10 days of storage. The response to MeJA treatments was dose-dependent and saturated, with the two highest doses giving similar responses. While the OTR-PE bags had an advantage over the control, the MeJA treatments were significantly better. These findings are in agreement with the study of Singh et al. (2014). Generally, bell peppers contain high levels of AsA, but the level declines during prolonged storage, especially during refrigerated storage (Gonzalez-Aguilar et al., 2004a; Sanchez-Bel et al., 2012). The loss of AsA in bell peppers could be due to the down-regulation of dehydroascorbate reductase (DHAR) by CI, which would impede the regeneration of AsA and cause its depletion from the bell peppers. Fung et al. (2004) found that MeJA treated bell peppers have increased ascorbate peroxidase (APX) activities, and this contributes to scavenging the reactive oxygen species (ROS), but APX utilizes AsA as a substrate. Potential cycling of these positive and adverse effects on AsA content may have caused the fluctuations seen in MeJA treated bell peppers in the present study. Time profiles of the free polyamines putrescine (Put), spermidine (Spd) and spermine (Spm) are shown in Fig. 6B-D. The Put and the Spd increased while the Spm decreased, consistently with storage time and across all the treatments. The actual treatments increased the levels and change rates of Put and Spd and reduced the loss of Spm in bell peppers, and the response to MeJA was dose-dependent. Overall the actual treatments increased the level of free polyamines during the study period. Gonzalez-Aguilar et al. (2000) observed that during cold storage bell peppers stored in PE bags showed higher levels of polyamines than the control. Polyamines are essential to plant regulators mainly involved in controlling plant senescence induced by abiotic stresses (Bitrian et al., 2012; Kaur-Sawhney et al., 2003). They interact with the phospholipids in the plant cell membranes to stabilize the membrane bilayer and to hinder membrane lysis and deterioration, otherwise caused by the chilling stress (Wang, 1993). Several studies report that exogenous treatment of plants with synthetic hormones and/or regulators could increase the biosynthesis of polyamines in plants (Wang et al., 2012; Zhang et al., 2012).

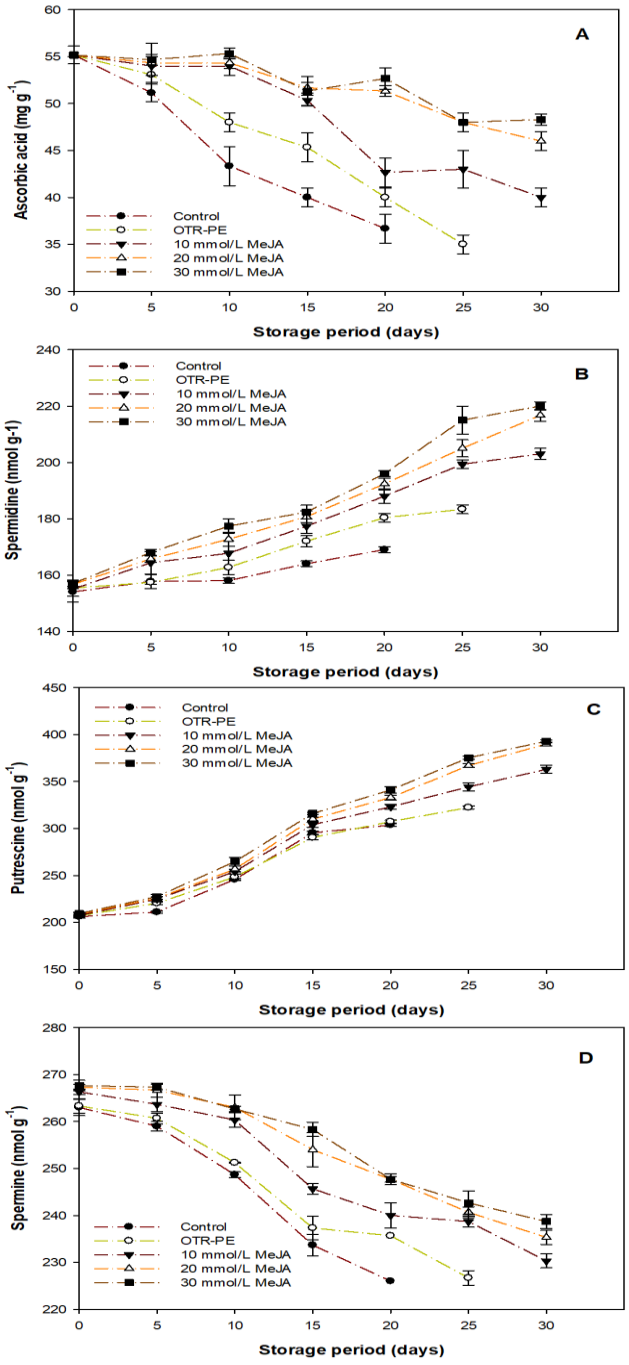

Fig. 6. Time profiles of ascorbic acid (A), and total polyamine content (B), for bell peppers stored at 4°C and 90% RH.

Cao et al. (2012) report that bell peppers had increased polyamine contents after exogenous treatment with the synthetic plant regulator 1-MCP, during refrigerated storage. Zhang et al. (2012) found that an exogenous MeJA treatment of cherry tomatoes increased the activity of endogenous arginine decarboxylase (ADC) during cold storage. The ADC is an enzyme contributing to the synthesis of polyamines in plants. Overall the prior literature corroborates our findings on the polyamine levels as reasonable.

4. Conclusions

The present study demonstrated that combining MeJA fumigation treatment with packaging in OTR-PE bags effectively controlled the chilling injury (CI) incidence in green bell peppers under prolonged refrigerated storage for up to 30 days. The combination treatment improved the resistance of bell peppers against CI, as indicated by increased polyamine level and by reduced CI index, reduced electrolytic leakage, and reduced loss of firmness. The barrier function of OTR-PE bags controlled moisture loss and maintained a headspace atmosphere during the storage, but combination treatments also including MeJA fumigation showed better protection of bell peppers against CI in a dose-dependent manner. The findings suggest that such combination treatments of fumigation and barrier packaging could efficiently and safely mitigate the risk of CI in bell peppers during refrigerated storage, at a low cost. The primary economic incentive is the significant improvement of shelf life.

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EVALUATE ANTI-BACTERIAL ACTIVITY OF *ROSMARINUS OFFICINALIS LINN.* EXTRACT AND *ORIGANUM SYRIACUM L.* ESSENTIAL OIL USING RAW CHICKEN MEAT

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ABSTRACT

The effect of oregano essential oil (OE) and rosemary extract (RE) on the survival and growth of *Salmonella enterica*, *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Enterobacteriaceae* (ENT), and the aerobic plate count (APC) in raw ground chicken meat stored at different temperatures. Five different treatments including i) control (no additives), ii) 150 ppm oregano essential oil (OE), iii) 350 ppm rosemary extract (RE), iv) 150 ppm OE + 350 ppm RE, and v) 14 ppm combination of butylated hydroxyanisole and butylated hydroxytolune (BHA/BHT) were prepared. All additives were showed significant ($P < 0.05$) antimicrobial activity (APC) compared to the control samples during storage time for both storage temperatures (4 and 8 ºC). However, the effect of OE was higher than RE depressing the growth of total aerobic bacteria. Similar effects were found, when the total viable count of ENT evaluated. The combined antimicrobial effect of RE and OE were significantly ($P < 0.05$) higher than using them alone, in both storage temperatures. Generally, all tested Food-borne pathogens (FBP) were significantly ($P < 0.05$) affected by adding OE and RE. Among all pathogens the combination of OE and RE were showed the highest treatment effect. Based on current results, it is concluded that both OE and RE had a potential antimicrobial activity, but it can be much stronger if incorporated together. In addition, this combination of OE (150 ppm) and RE (350 ppm) may represent a prospective natural replacement to the synthetic antimicrobial currently used in the meat industry.

1. Introduction

According to the recent estimation of the American Center for Disease Control and Prevention (CDC), 48 million people get sick, 128,000 are hospitalized, and 3000 die yearly by food-borne diseases (FBD) (CDC, 2017). In addition, the U.S Department of Agriculture (USDA) estimates that food-borne illnesses cost $15.6 billion each year. In contrast, FBD are likely to increase with the consumption of contaminated food, and increased with the value chain in low and middle income countries (Grace, 2015). For instance, in Jordan, several food illnesses occurred without reporting to the Jordan Food and Drug Administration (JFDA) or their Ministry of Health. This due to weakness in covering all of these report issues daily, poor equipment's, and difficulty to diagnose fast. However, preventing of FBD would have a positive effect economically, and on the overall human health. In addition, it will decrease hospitalization cost, protect people and saving life. The US-CDC

summarizes two major groups of food-borne illnesses: 1) Known Food-borne pathogens (FBP) (31 pathogens) and 2) Unspecified agents (agents not yet identified). Generally, many pathogens are associated with raw meat cause of human FBD as Escherichia coli O157:H7, Salmonella enterica, and Listeria monocytogeneses which consider the most important (AMSA, 2017). These pathogens can grow fast, transferred in raw meat, if poor hygienic system and insufficient cooking temperature had been available. Meats consider a rich media for these pathogens (e.g. vitamin, mineral, pH, and amino acid) support their growth rate, and proliferation (Mead, 2004).

Researchers and meat industry are still willing to prevent or delay the growth of these pathogens in various meat products. However, they usually use synthetic antimicrobial additives such as nitrate or nitrite, butylated hydroxyanisole (BHA), butylated hydroxyltolu-ne (BHT) which used to decrease microbial growth, and extend meat products shelf life (Turcotte and Saheb, 1978; Sebranek and Bacus, 2007; Ahn et al., 2007; Kumar et al., 2015). On the other hand, these synthetic antioxidants may have toxicological and carcinogenic effect on human tissues (Sebranek and bacus, 2007; Oostindjer et al., 2014). Thus, finding potential antimicrobial replacement without affecting food quality or their nutritive values is the most challenging topic. This approach is growing fast due to the increase in consumer demand on natural and organic meat products. Essential oils, herbal plants extracts (e.g thyme, mint, sage, oregano, and rosemary), and plant antimicrobial compounds were extensively studied for this purpose (Kumar, 2015; Hintz et al., 2015).

Oregano essential oil contains two important phenols: carvacrol and thymol, which constitute about 78-82% of its composition, and represent most of their antioxidant activity (Adam et al., 1998; Yanishlieva et al., 1999). In addition, they are the main components of the OE responsible of their antimicrobial activity (Ultee et al., 2002; Nostro et al., 2004; 2007). Sivropoulou et al. (1996) study three species of oregano essential oils and they found strong antimicrobial activities against eight different strains of gram-positive and negative bacteria. Chouliara et al. (2007) reported that adding OE on fresh chicken meat using modified atmosphere packaging extend shelf life, and significantly decrease their microbial populations (lactic acid bacteria, TVC, pseudomonas spp., yeast). Thymol and carvacrol damaged bacterial cell membranes, destabilized the operation of phospholipids bilayer of bacteria, and changed internal cell processes (Kalemba and Kunicha, 2003; Cristani et al., 2007; Xu et al., 2008; Di Pasqua et al., 2010).

The antimicrobial activity of OE is well documented, and reported as effective natural antimicrobial for many FBP such as Salmonella typhimurium, Staphylococcus aureus, Vibrio parahaemolyticus, Listeria monocytogenes, and Escherichia coli (Sivropo uluo et al., 1996; Ozcan, 2001; Lin et al., 2004).

The antioxidant/antimicrobial activity of RE linked with their content of phenolic diterpenes such as carnosic acid, carnosol, rosmanol, rosmariquinone and rosmaridiphenol, ursolic acid, and caffeic acid (Aruoma et al., 1992; Basaga et al., 1997). In addition, RE has been reported to have anti-inflammatory, anti-oxidant, and anti-cancer properties (Moore et al., 2016). However, carnosic acid and carnosol represent 90 % of the antioxidant properties of RE (Aruoma et al., 1992; Erkan et al., 2008). In addition of their antioxidant activity, several authors have been reported RE as antimicrobial additive because of its phenolic constituents, and their ferrous ion-chelating effect (Zhang et al., 2016; Yin et al., 2016). However, carnosic acid, carnosol, and rosmarinic acid may be the main bioactive antimicrobial compounds present in RE (Campo et al., 2000; Mereno et al., 2006; Jiang et al., 2011; Issabeagloo et al., 2012). For example, Riznar et al. (2006) who tested the antimicrobial activity of RE in chicken meat Frankfurters found that the prepared oil extract possess antimicrobial activity using aerobic plate count (APC) technique. In addition, the

extract was reported to have wide range of antimicrobial effect against several food-borne illness strains (Hussain et al., 2010; Kahraman et al., 2015). Furthermore, it enhances the efficiency of synthetic antibacterial activity such as BHA which allows using lower amount of these additives (Romano et al., 2009). However, among all the studies conducted before RE was not been deeply characterized of their antimicrobial effect using ground meat system. To the best of our knowledge, there were no research study investigate the antimicrobial effect of Rosmarinus officinalis Linn. extract and Origanum syriacum L. essential oil, in combination and their recommended level, on ground chicken meat. In addition, this combination may have synergistic effect depending on many factors such as media (meat composition), pH, free radicals, enzymes, metabolic of bacteria, and their cell structure. Adding these natural preservative in combination (hurdle technology) with other synthetic antimicrobial enhance the barrier against FBP (Davidson et al., 2005).

Thus, the aim of this study was to investigate the effect of OE (Origanum syriacum L.) or RE (Rosmarinus officinalis Linn.), alone or in combination, on the survival and growth of Escherichia coli O157:H7, Salmonella enterica, Listeria monocytogeneses, aerobic mesophilic bacteria and Enterobacter-iaceae in raw ground chicken meat stored at different temperatures.

2. Materials and methods

2.1. Meat Patties preparation

Fresh chicken thigh meat was purchased from a local grocery market and double ground a through a 8-mm plate then a 3-mm plate (Moulinex, Type DKA1, France). Prepared treatments were including: i) Control (no additive), ii) 150 ppm OE, iii) 350 ppm RE, iv) Combination (CM) of 150 ppm OE + 350 ppm RE, and iv) 14 ppm combination of butylated hydroxyanisole/butylated hydroxytol- une (0.02 % of BHA/BHT based on the fat content) were prepared. Oregano essential oil (OE) and RE levels were selected depending on previous meat quality studies considering the maximum antioxidant activities (Data were not shown). Oregano essential oil (*Origanum syriacum* L. pure extract) was obtained from a certified company in Jordan (Green Fields Factory for oils, Amman/Jordan) using the most efficient purification, extraction, and steam distillation methods. The HPLC analysis of the OE indicated that 76.39% of the essential oil was carvacrol. Rosemary pure extract (Cultivated in Jordan) was obtained from same company source, and the HPLC analysis of the RE was measured (Method of Okamura et al. 1994), and it was containing 26 ± 3% as the average of phenolic diterpenes (4 % carnosol and 6 % carnosic acid and other phenolic constituents). The BHA/BHT powder, RE, and OE were dissolved in 10 ml of 100 % ethanol, and then mixed with 50 ml mineral oil (Sant Cruz Biotechnology, Dallas, TX, USA), to prepare their stock mixture. The ethanol mixed with mineral oil was split out using a rotary evaporator (Heidolph, Model Laborota 4001-effecient) at (70 °C, 175 mbar vacuum pressure) before adding the stock to the meat mixture. Each treatment additive was added to the ground meat, and then mixed for 3 min in a bowl mixer. All treatments were added with the same quantity of mineral oil to get the same conditions. Chicken meat patties (approx. 25 g each) were individually packaged in separate oxygen-permeable bags (Polyethylene, Size : 11 x 25 cm, Future for Plastic Industry , Al-Moumtaz bags, Co. L.T.D, Amman, Jordan), and used for the raw-meat study performed in the Microbiology laboratory of Agriculture Collage, at Mutah University. Ground meat samples were placed in a cold condition (4.5 ^{o}C) and used in experiments within 2-3 hours.

2.2. Water Activity and pH

Water activity (a_w) of raw ground meat was measured using water activity meter (Rotronic HP23-AW-A-SET-40, Portable Analyzer, USA). Meat sample was filled to the line of each disposable cup (Non-inoculated raw ground meat) and spread on the surface of their

container. After calibration, meat sample was placed inside the container and water activity value recorded. The Ultimate pH values of the chicken raw meat samples were determined using a pH meter (PL-600, pH/mV/Temp Meter, Taiwan) after homogenizing the 1.0-g samples with 9 ml deionzed distilled water (DDW) (Sebranek et al., 2001).

2.3. Bacterial Cultures Preparation

Four-strains each of *Listeria monocyte-genes, Salmonella enterica*, and *Escherichia coli* O157:H7, were obtained from the culture collection of the Microbial Food Safety Laboratory at Mutah University, and JFDA (Jordan Food and Drug Administration) laboratory. Frozen stocks (in 10 % glycerol at -80 ºC) were thawed and cultured separately in tryptic soy broth (TSB; Biolab Zrt. 1141 BUDAPEST Öv u. 43, Hungary) supplemented with 0.6 % yeast extract (TSBYE) at 35 ºC for 24h. Two consecutive 24-h transfers of the each culture were prepared in TSBYE (35 ºC) to make our working cultures. Before the inoculation process each working culture was individually grown in 10 mL of TSB supplemented with 0.6 % yeast extract (TSBYE- Biolab Zrt. 1141 BUDAPEST Öv u. 43, Hungary) at 35 ºC for 24h. These strains were adapted gradually in the TSBYE with different concentrations of added Naldixic acid (NA; antibiotic; M.W 232.24, 10 ug/ml, 30 ug/ml, and 50 ug/ml; Santa Cruz Biotechnology, Dallas, TX, USA). Two consecutive transfers 24-h for each Naldixic acid resistant strain (NAR) cultures were prepared. For each pathogen, when starting each study, 6 ml of each individual NAR adapted culture in TSBYE was aseptically transferred to a sterilized centrifuge tube to give a total 24 ml of strain mixture culture (NARC).

Bacterial cells from each mixture of strains were harvested by centrifugation at 10,000 x g for 10 min at 4 °C (Medical Centrifuge, TG16G; Hunan Kaida Scientefic Instruments Co., Ltd; China). The pelleted cells were re-suspended in a 24-ml sterilized saline (0.85 % NaCl), washed by vortexing, and then centrifuged again at the same speed and temperature conditions. After harvesting the cells from the second centrifugation, the collected cells were suspended in fresh saline (0.85% w/v NaCl) and diluted (10-fold) using tubes of saline to obtain 106 CFU/ml in a suspension of washed cells used for inoculating the ground chicken meat.

2.4. Preparation and Inoculation of Meat Samples

Ground meat samples were transported to the Microbial Food Safety Laboratory (Dept. of Food Science & Human Nutrition) for inoculation and microbial analysis. Each sample was inoculated with a mixture of four nalidixic-acid resistant serotypes of *Salmonella enterica* (*S. Typhimurium, S. Newport, S. Kentucky, S. Oranienburg*), 4-strain mixture of *Escherichia coli* O157: H7 (ATCC 35150, ATCC 43894, ATCC 43895, WS 3062) and 4-strain mixture of *Listeria monocytogenes* (Scott A, H7596, H7762 and H7962; all serotype 4b) to give an initial cell concentration of ~104 colony forming units (CFU)/g for each pathogen. All inoculated packages of ground chicken meat were closed, manually massaged for 40 s from outside of the bag, and held at different storage times and temperature. At set intervals during storage, samples of raw meat were analyzed for *Salmonella, Escherichia coli* O157:H7 and *Listeria monocytogenes* survivors. In addition, separate bags of non-inoculated ground meat were stored at 4 and 8 °C and analyzed for aerobic plate counts, and numbers of viable *Enterobacteriaceae*.

2.5. Microbial Analysis

Ground chicken patties were aseptically opened from their packages, and two-25 g portions of meat were transferred into separate sterile filter-lined stomacher bags (Seward^{Tm}). Sterile 0.1% (w/v) peptone water (225 ml) was added to each bag. The meat mixture was homogenized (60 second) in a laboratory stomacher blender (Easy Mix, AESAP1068; 35172 BRUZ, France) programmed at medium

speed. After that, serial dilutions of meat samples (10-fold) were prepared in sterilized tubes of 0.1% peptone and 0.1 ml aliquots of diluted samples were surface plated on appropriate selective agar media to enumerate pathogenic bacteria.

Sorbitol MacConkey agar (SMA), Modified Oxford (MOX) agar, and Xylose lysine Deoxycholate XLD agar were used for: *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella enterica*, enumeration respectively. Inoculated agar plates were incubated at 35 ºC and bacterial colonies total count were measured after 48 hours. The aerobic plate count (APC) was determined by surface plating aliquots (0.1 ml) of meat homogenate on tryptic soy agar (TSA), incubating the inoculated TSA plates at 35 ºC, and counting bacterial colonies at 48 hours. The numbers of viable *Enterobacteriaceae* (ENT) were determined using the agar overlay method. Aliquots (0.1 ml or 1.0 ml) of meat homogenate were placed in a petri dish and molten TSA (48 ºC) was poured onto the dish to form the first layer. The TSA plate was held for 60 minutes at ambient temperature before pouring a layer of violet red bile agar (VRBA) over the TSA. After TSA/VRBA media solidified in all plates, it was incubated at 35 ºC and bacterial colonies were counted at 24 hours.

2.6. Statistical Analysis

Completely randomized design (CRD) was performed in this study. Two separate samples per treatment per replication were analyzed over 2 replications of each independent experiment. The statistical analysis was performed using the procedures of generalized linear model (Proc. GLM, SAS program, version 9.3, 2012). Mean values and standard error of the means (SEM) were reported. The significance was defined at $P < 0.05$ and Tukey test or *Tukey's Multiple Range* test were used to determine whether there are significant differences between the mean values.

3. Results and discussion

In general both total aerobic (TA) and ENT count used to evaluate food contamination, manufacture system hygiene, quality and food safety (American public Health Association, 1984). In addition, *Salmonella, Campylobacter, Staphylococcus aureus, Escherichia coli* O157:H7, *Clostridium perfringens*, and *Listeria monocytogenes* are considered the most known FBP identified in the United State by the Center for Disease Control and Prevention. These also are on the list of major FBP in most of low income countries such as Middle East, Africa and South Asia countries.

There were no significant ($P > 0.05$) effects by adding RE, OE, BHA/BHT, and CM treatments on the initial pH values at day 0 (Table 1). In addition, the pH values were stable, and the average was the same during storage time using random samples from each treatment (data not shown). Average water activity (a*w*) values (0.98) were similar among all treatments, and no significant differences ($P > 0.05$) appeared. Thus, mixing both RE, OE, and (BHA/BHT) with ground chicken meat had no effect on their pH value or their water activity. Therefore, we had similar intrinsic condition which avoid any variation could happen due to the acidity or a*w* during storage time.

Part of the ground chicken (thigh) meat samples were stored at a temperatures of 4 ºC which represent the absolute refrigeration temperature (4ºC), and other were stored in 8 ºC as an example of abuse temperature. There were no significant differences ($P > 0.05$) between all treatments using the (APC) test of both storage temperatures at day 0 (Table 2). However, a significant ($P > 0.05$) antimicrobial effect was appeared at day 2 among all treatments compared to the control samples. It was reported that adding OE at level 0.1% to the refrigerated (4 ºC) ground chicken breast meat enhances their shelf-life (Chouliara et al., 2007). In addition, mixing the OE (0.5 % and 1%) decrease the total microbial growth in

ground beef placed under refrigeration condition (5ºC) (Skandamis and Nychas, 2001). However, the antibacterial effect of OE separately was significantly higher than the RE at day 2 using 4ºC of storage temperature. Carvacrol and thymol are the two major antimicrobial agents in OE responsible on their antimicrobial activity (Sivropoulou et al., 1996). In addition, RE have antimicrobial activity due to their phenolic composition (Azizkhani and Tooryan, 2015; Zhang et al., 2016). On the other hand, no significant differences ($P > 0.05$) were found between both treatments (OE and RE) at day 2 using temperature of 8 ºC. However, the combination (OE and RE) effect at day 2 was significantly ($P < 0.05$) higher than using them alone, and by testing both temperatures. In addition, no significant differences ($P > 0.05$) were found using BHA/BHT additive compared to the combination treatment at day 2. At day 4 of storage time the total bacterial count increased significantly ($P < 0.05$) compared to the total count at day 2 among all treatments. Similar results were found using both temperatures where the OE showed higher significant antibacterial effect compared to the RE at day 4 of storage time. However, the highest antimicrobial effect (day 4) was found using the combination treatment (OE and RE) compared to the other treatments additives. This was in agreement with Al-Hijazeen (2014) who investigates the effect of adding different level of OE (*Origanum vulgare* subsp. *Hirtum*) on the APC and other Food-borne pathogens in a ground chicken meat. This antimicrobial activity was continued until day 8 of storage time under 4 ºC of refrigeration conditions. There were no significant differences ($P > 0.05$) appeared between both treatments (BHA/BHT) and combination (OE and RE) at day 8 of storage time using 4 ºC. The antimicrobial activity of CM (RE+OE) treatment depresses the total APC (Log cfu/g) by 2.28 colony unit compared to the control treatment at day 8 of storage time.

Table 1. Water activity and pH values of raw ground chicken (thigh) meat at day 0.

Sample	a_w	**pH value**
Control	0.981^{a}	6.156^{a}
Oregano (OE)	0.982^{a}	6.163^{a}
Rosemary (RE)	0982^{a}	6.105^{a}
Combination(OE+RE)	0.982^{a}	6.124^{a}
BHA/BHT	0.980^{a}	6.127^{a}
SEM	0.003	0.038

$^{a-c}$ Statistically significant differences ($P < 0.05$) between treatment.
N = 4 for water activity and 3 for pH value.
Treatments: T1) Control; T2) 150 ppm OE; T3) 350 ppm RE; T4) Combination of OE & RE: T5) 14 ppm of (BHA/BHT).

Table 2. Total Plate Counts of raw chicken thigh meat during storage at 4 °C and 8 °C.

Treatments	**Storage Time**					**SEM**
	0 day	2 days	4 days	6 days	8 days	
	---------- Log CFU/g meat ----------					
Storage at 4°C						
Control	4.10^{az}	5.53^{ay}	7.80^{ax}	8.42^{aw}	9.56^{av}	0.090
Oregano (OE)	4.20^{az}	4.84^{cy}	6.63^{cx}	7.41^{cw}	7.88^{bcv}	0.053

Rosemary (RE)	4.15^{az}	4.15^{by}	7.11^{bx}	7.60^{bw}	8.10^{bv}	0.043
Combination (OE+RE)	4.15^{az}	4.45^{dy}	6.25^{dx}	6.87^{ew}	7.28^{dv}	0.045
BHA/BHT	4.16^{az}	4.62^{cdy}	6.42^{dx}	7.08^{dw}	7.54^{cdv}	0.033
SEM	0.027	0.067	0.046	0.039	0.084	
Storage at 8 °C						
Control	4.20^{az}	7.02^{ay}	8.51^{ax}	-1	-	0.068
Oregano (OE)	4.18^{az}	6.34^{by}	7.61^{cx}	-	-	0.023
Rosemary (RE)	4.23^{az}	6.55^{by}	7.90^{bx}	-	-	0.040
Combination (OE +RE)	4.18^{az}	5.75^{cy}	6.74^{ex}	-	-	0.028
BHA/BHT	4.20^{az}	5.91^{cy}	6.99^{dx}	-	-	0.033
SEM	0.025	0.058	0.034			

[1]Not determined. n=4.
[a-e]Different superscripts within a column differ significantly ($P < 0.05$).
[x-z]Different superscripts within a row differ significantly ($P < 0.05$).
Treatments: T1) Control; T2) 150 ppm OE; T3) 350 ppm RE; T4) Combination of OE & RE: T5) 14 ppm of (BHA/BHT).

The *Enterobacteriacea* total count of control samples was the highest (8.52 Log_{10} CFU/g) at day 8 of storage time (4ºC) compared to the other treatments. However, the ENT total count increased rapidly using higher temperature (8°C) and finished with 7.87 Log_{10} CFU/g at day 4 of storage time. There were no significant ($P > 0.05$) differences of ENT viable count among all treatment at day 0 of storage time. However, all treatments additives were showed significant ($P < 0.05$) antimicrobial activity against ENT (Gram negative bacteria) at day 2 of storage time using both temperatures. In addition, the combination and (BHA/BHT) treatments were showed higher significant ($P < 0.05$) anti-bacterial activity reduced the growth of ENT compared to the OE or RE alone at day 2 of storage time. The combination treatment (OE+RE) showed the highest significant ($P < 0.05$) antibacterial activity among all additives at day 4 using both temperatures. In addition, OE showed higher significant ($P < 0.05$) anti-ENT effect compared to the RE using previous temperatures at day 4. The effect of using OE against ENT growth was reported by several researchers (Al-Hijazeen, 2014; Boskovic et al., 2015; Swamy et al., 2016). Furthermore, the (BHA/BHT) additive showed higher antimicrobial activity against ENT compared to the OE or RE alone. In addition, the combination treatment showed the highest significant ($P < 0.05$) effect through the period from day 6 to 8 using 4ºC. Furthermore, the synergistic effect (Combination) of many plant extract had been reported to give antimicrobial activity better than using them separately (Pol and Smid, 1999; Lin et al., 2004; Brewer., 2011; Abd El-Kalek and Mohamed, 2012). However, despite of the significant antimicrobial effect of the combination treatment, no synergistic effect were appeared. All tested pathogenic microbes (FBP) were having close total bacterial count at day 0 of storage time. In previous preliminary study it shows slow growth rate using these pathogenic at temperature less than 4 ºC (*data were not shown*). Overall, pathogens were showed significant antibacterial activities reduce the growth of *Escherichia coli O157:H7, Listeria monocytogenes,* and *Salmonella enteric* in all treatments additives compared to the control samples after day 2 of storage time. The antimicrobial activity of OE (Chouliara et al., 2007; Zhang et al., 2016) and RE (Kahraman et al., 2015) examining into the meat system

against FBP are well documented. The Antimicrobial effect of adding RE (350 ppm) was significantly ($P < 0.05$) higher than OE (150ppm) on the total viable count of *Escherichia coli O157:H7* at day 4 and 6 of storage time. However, the highest significant effect against *E.coli* growth was by using the combination additive (RE + OE) compared to the other treatments at day 6 of storage time. Xu et al., 2008 reported that the antimicrobial effect of oregano oil on the growth of *E.coli* O157:H7 is due to their high content of carvacrol.

Table 3. Numbers of viable *Enterobacteriaceae* in raw ground chicken meat stored at 4 °C and 8 °C.

	Storage Time					
Treatments	0 day	2 days	4 days	6 days	8 days	**SEM**
	---------- Log CFU/g meat ----------					
Storage at 4 °C						
Control	4.18^{az}	5.34^{ay}	6.56^{ax}	7.90^{aw}	8.52^{av}	0.026
Oregano (OE)	4.15^{az}	4.67^{cy}	5.31^{cx}	6.70^{bw}	7.55^{cv}	0.033
Rosemary (RE)	4.21^{az}	4.80^{by}	5.51^{bx}	6.83^{bw}	7.73^{bv}	0.026
Combination (OE+RE)	4.16^{az}	4.47^{dy}	5.00^{ex}	6.04^{dw}	6.91^{ev}	0.031
BHA/BHT	4.17^{az}	4.54^{dy}	5.15^{dx}	6.31^{cw}	7.17^{dv}	0.026
SEM	0.019	0.023	0.027	0.040	0.029	
Storage at 8 °C						
Control	4.24^{az}	6.53^{ay}	7.87^{ax}	-1	-	0.024
Oregano (OE)	4.29^{az}	5.18^{by}	6.41^{cx}	-	-	0.025
Rosemary (RE)	4.26^{az}	5.25^{by}	6.81^{bx}	-	-	0.377
Combination (OE+RE)	4.22^{az}	4.75^{dy}	5.70^{ex}	-	-	0.018
BHA/BHT	4.24^{az}	4.93^{cy}	6.21^{dx}	-	-	0.025
SEM	0.022	0.033	0.024			

[1]Not determined. n=4.
[a-e]Different superscripts within a column differ significantly ($P < 0.05$).
[x-z]Different superscripts within a row differ significantly ($P < 0.05$).
Treatments: T1) Control; T2) 150 ppm OE; T3) 350 ppm RE; T4) Combination of OE & RE: T5) 14 ppm of (BHA/BHT).

During this study (day 2-8) the OE showed higher significant ($P < 0.05$) effect against the growth of *Listeria monocytogenes*. The Anti-*Listeria monocytogeneses* effect of adding OE also reported using fish and other meat system (Lin et al., 2004). Carvacrol and other phenolic compound can affect the potential bacterial cell membrane and the inside biological activities (Ultee et al., 2002; Di Pasqua et al., 2010). However, both treatments (Combination and BHA/BHT) showed the highest antimicrobial effect compared to the other additives. In addition no significant differences appeared between the two treatments at day 6 and 8 of storage time.

Salmonella enteric count was significantly ($P < 0.05$) higher in meat samples treated by OE compared to RE treatment samples during the experimental period. This indicates that the incorporation of RE (350 ppm) was very effective against *Salmonella enteric* survival in ground chicken meat. Kahraman et al., 2015 reported that adding 0.2 % RE (Aegean region of Turkey) to poultry meat fillets did not reduce the growth rate of *S. Typhimurium or L. monocytogenes* at 4°C stored for 7 day. However, many factors could affect their

antimicrobial activity (RE) such as extraction method, plant genetic variation, bacterial structure and strains, oil composition, and the method of mixing plant extract into the food system. In addition to its strong antioxidant activity, RE had been reported to have antimicrobial effect on the growth of various FBP tested in the lab (Smith-Palmer et al., 1998; Hammer et al., 1999). It had been reported that adding RE or their essential oils have a positive effect retarding microbial growth of both Gram-positive and Gram-negative bacteria in different meat systems (Georgantelis et al., 2007; Liu et al., 2009; Jiang et al., 2011). The antimicrobial mechanism of these plant extracts, or their essential oils; explained by their effect on the bacterial cell membrane integrity and over all permeability (Ojeda-Sana et al., 2013).

Table 4. Number of viable *Escherichia coli O157:H7, Listeria monocytogenes*, and *Salmonella enterica* in ground raw chicken meat during storage at 8 °C.

		Storage Time				
Treatments	0 day	2 days	4 days	6 days	8 days	**SEM**
---------- Log CFU/g meat ----------						
Escherichia coli						
Control	4.09^{az}	5.22^{ay}	6.54^{ax}	8.38^{aw}	-1	0.022
Oregano	4.11^{az}	5.04^{by}	6.13^{bx}	7.38^{bw}	-	0.048
Rosemary	4.13^{az}	4.88^{by}	5.75^{cx}	7.07^{cw}	-	0.046
Combination	4.08^{az}	4.38^{dy}	5.21^{dx}	6.62^{ew}	-	0.032
BHA	4.15^{az}	4.60^{cy}	5.63^{cx}	6.85^{dw}	-	0.033
SEM	0.042	0.04	0.037	0.031		
Listeria monocytogenes						
Control	4.16^{az}	5.24^{ay}	6.47^{ax}	7.86^{aw}	8.57^{av}	0.043
Oregano	4.16^{az}	4.61^{cy}	5.52^{cx}	6.81^{cw}	7.63^{cv}	0.045
Rosemary	4.19^{az}	4.89^{by}	5.91^{bx}	7.09^{bw}	7.95^{bv}	0.028
Combination	4.20^{az}	4.44^{dy}	4.83^{ex}	6.17^{dw}	6.85^{dv}	0.03
BHA	4.18^{az}	4.46^{cdy}	5.05^{dx}	6.32^{dw}	6.98^{dv}	0.031
SEM	0.0144	0.036	0.049	0.041	0.031	
Salmonella enterica						
Control	4.13^{az}	5.55^{ay}	6.93^{ax}	8.19^{aw}	-	0.036
Oregano	4.17^{az}	5.32^{by}	6.16^{bx}	7.60^{bw}	-	0.023
Rosemary	4.16^{az}	5.08^{cy}	5.72^{cx}	7.07^{cw}	-	0.034
Combination	4.17^{az}	4.63^{dy}	5.11^{dx}	6.73^{dw}	-	0.021
BHA	4.20^{az}	4.68^{dy}	5.25^{dx}	6.83^{dw}	-	0.037
SEM	0.036	0.025	0.036	0.024		

[1]Not determined. n = 4.
[a-e]Different superscripts within a column differ significantly ($P < 0.05$).
[x-z]Different superscripts within a row differ significantly ($P < 0.05$).
Treatments: T1) Control; T2) 150 ppm OE; T3) 350 ppm RE; T4) Combination of OE & RE: T5) 14 ppm BHA/BHT.

In addition, both treatments (CM and BHA/BHT) showed the highest significant anti-salmonella effect during the experimental period compared to the other additives. Generally, mixing RE with meat samples extends their shelf life, and if used in combination their effect would be maximized (Azizkhani and Tooryan, 2015). However, no significant differences ($P > 0.05$) between both treatments (BHA/BHT and CM) appeared at all interval time.

4. Conclusion

Both OE (150ppm) and RE (350ppm) were showed significant antimicrobial effect ($P < 0.05$) against APC, ENT, and all selected food-borne pathogens compared to the control samples tested. However, their combination (OE + RE) effect is higher than using them separately. Overall, their antimicrobial effect may consider very comparable compared to the synthetic one; however no synergistic effect appeared. This combination of RE and OE could be a good promising in the future of meat processing industry. In addition, it could be a good natural replacement or partial substitution to the synthetic one.

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OPTIMIZING OF QUINCE PEEL EXTRACTION BY USING SOLVENT BASED ON ANTIOXIDANT POWER AND LUTEIN CONTENT

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ABSTRACT.
Food waste and non-edible parts of fruits include functional compounds such as antioxidants. The present study investigates the optimal temperature, time, and amount of solvent in extracting antioxidant extract of quince peel with two solvents of N-Hexane and Ethyl acetate based on FRAP, DPPH and total phenol methods and quantity of Lutein with the Response Surface Methodology (Central Composite). The results showed that increasing the solvent concentration in all experiments led to improved extraction of antioxidant compounds and enhanced antioxidant power of the extracts. However, due to the oxidation of carotenoids, phenol polymerization, and maybe the extraction of impurities in high levels of temperature and time, the antioxidant power of the extracts had relatively reduced. The best extraction condition with ethyl acetate was in 56.25 ºC, 3.6 h, and concentration of solvent 35 times of quince peel powder and for hexane solvent in 50.56 ºC, 3.6 h, and concentration of solvent 35 times of quince peel powder which was achieved by Minitab Software. The most optimal amount of Lutein was achieved in extracting with ethyl acetate in 50ºC, 5 h, and solvent 30 times of powder, therefore, the best amount of Lutein in extracting with hexane in 50ºC, 2 h, and solvent 30 times of quince peel powder. In general, the extraction of ethyl acetate was more successful in achieving antioxidant extract.

1.Introduction

These days because of the importance of waste management in various industries such as food industry, there has been a particular attention to reusing waste processing industry in order to increase their added value and the extraction of functional compounds for other food industry departments has also increased. In recent years, there has been considerable attention to advantages of antioxidants existent in non-food parts of the fruits. Processing fruits, vegetables, and oil seeds have produced a lot of waste which include fruit peel, seeds, and pits. While in developed countries, waste is considered as a valuable resource for the recycling different enriched products, supplements and additives (Ranjbar, et al., 2011; Socaciu, 2008).

Antioxidants are one of useful compounds used in different food products and in high-fat products in particular. In addition to their helpful features, antioxidants postpone the start of the oxidation of oils and fats and reduce the production of free radicals. The conducted studies show that some synthetic antioxidants have unpleasant physiological effects on humans that are the reason of the increasing interest in extraction of natural antioxidants, especially in the last two decades (Sharifi et al., 2009; Mahdavi, et al., 1995).

Carotenoids and phenol compounds are among the compounds that not only make a

better color and taste in fruits and vegetables, but also have antioxidant features that are abundant in fruit peels and vegetable waste.

There have been a lot of studies on using solvents and different extraction conditions for maximizing extraction of antioxidant extracts from fruits and vegetables. Mokrani, et al. (2016) conducted a study for merging effects of the type of solvent (ethanol, methanol, acetone, and water), acetone concentration, acidity of the solvent, time and temperature on extracting all phenol compounds, flavonoid compounds and antioxidants in peach fruit; they concluded that all conditions of the study had a significant impact on the amount of extraction of these compounds. For investigating Arabshahi and Orouj (2007) used three solvents of methanol, water, and acetone in order to investigate antioxidant features of berry leaves and it showed that methanol extracted the greatest amount of phenol compounds (9.32 g of acid Gallic per 100 g of dry extract). Singh et al. (2002) used water, methanol, and ethyl acetate for extracting phenolic compounds from the peel and seeds of pomegranate; in this study, methanol had the highest efficiency.

Regarding these, quince peel can be considered as a food waste. Quince fruit has dried meat and is fluffy with a sour and almost astringent taste. Quince is full of vitamins A and B, Calcareous minerals and tannin. Its anti-cancer property is due to the presence of compounds full of antioxidants. The total amount of phenol compounds in the fruit has been reported as 119 mg/kg (Tzanakis et al., 2016). Therefore, this study examines the optimal conditions for extracting antioxidant extract of quince peel by two solvents of n-hexane and ethyl acetate. The reason for using these two solvents is their similar polarity to phenolic compounds and carotenoids.

2.Materials and Methods

In this study, the extraction of antioxidant extract of quince peel was employed by two solvents of n-hexane and ethyl acetate. The antioxidant powers of these extracts were scrutinized by three methods of DPPH, FRAP, and total phenol. And finally, the amount of extracted Lutein was examined with HPLC as a healthy carotenoid.

2.1. Preparing Extracts

When quince peel was dried in a vacuum oven at 40 ° C for 14-13 hours at a pressure of 70 mbar and powdered with laboratory mills, 10 g of the powder was weighed and transferred to a 500-ml Erlenmeyer flask. Then, the intended solvent was added to it in amounts of 10 to 30 times of dried quince powder and the Erlen was placed in a shaking incubator in the range of 25 to 50 °C according to boiling point of the solvents (boiling point of ethyl acetate is 77 ° C and boiling temperature of hexane is 69 ° C) and also the least adverse effect on the antioxidant feature of extracts; the extraction which could take 2-5 hours. Two solvents of n-hexane and ethyl acetate were separately utilized in this study. When the intended time passed, the obtained extract was cleared by the Whatman filter paper, and eventually the left solvent of the extracts was removed at 40 ° C in the evaporator rotary under vacuum at a speed of 70 rpm under reduced pressure until its weight became constant. After that, the extracts were transferred into a container with aluminium cover and for presenting unpleasant changes in its antioxidant feature; it was placed in a dark environment (Rojas et al., 2001; Sheikhzadeh et al., 2014; Naghavi et al., 2014).

2.2. Measuring the Antioxidant Power

2.2.1. Assessing antioxidant capacity with DPPH radical

This method is based on reduction of diphenyl picryl hydrazyl (DPPH) free radical by antioxidants in the absence of other free radicals in the environment that results in creating a color in the environment, whose intensity is measurable by spectrophotometry (Prevec et al., 2013). By measuring the absorption of samples at a wavelength of 518 nm compared to that of the control group and by using equation 1 - 3 free radical scavenging

(I%) of each extract was determined (Saha et al., 2004).

I % = (A control-A sample) /A control × 100

A control is the absorbance of control at 518 nm

A sample is the absorbance of sample at 518 nm

2.2.2. Assessing antioxidant capacity by ferric reducing antioxidant power (FRAP) method

An oxidation-reduction reaction (redox) is used in FRAP method which is accompanied by change of color. When the regenerative reaction (antioxidant) donates its electron, some colorful material is produced and the intensity of the produced color can be easily measured which shows the reaction progress (Guo et al., 2003; Martinek, 1968). When the antioxidants are present, the intensity of blue color increases in the environment and by measuring the intensity of the color, the antioxidant capacity can be explored (Liu et al., 1982).

2.2.3. Determine the area under the curve of pure Lutein

For recognizing Lutein, first pure Lutein was dissolved in a solvent including methanol (80%), distilled water (5%), methyl tert-butyl alcohol (15%) and then was poured into the reservoirs for the mobile phase HPLC device until the device reaches the ground state (Rodriguez et al., 1998). Then 10 ml of the prepared purified extract Lutein at 245 nm wavelength with a flow rate of 1 ml per minute was injected into the HPLC system, regardless of the peak related to its solvent after 2.243 minutes, the first and the highest peak was considered as the standard chromatogram for Lutein (Skoog, D.A et al., 2014).

2.3. Statistical Analysis

In this study, the effect of three parameters of extraction temperature (range 25 to 50 ° C), extraction time (2 to 5 hours), and the amount of solvent (10 to 30 times of quince peel powder) the antioxidant properties has been investigated in the Response Surface Methodology by central composite Minitab software and the optimum conditions of extraction was introduced based on antioxidant power of each solvent. The designed experiments with this method and by different levels of temperature, time, and solvent is shown in Table 1.

Due to high number of treatments and costs of HPLC method, the amount of Lutein in extracts was evaluated only in two levels of time, temperature, and solvent concentration.

Table 1. Experimental runs, independent variable levels and replicate runs in RSM design by central Composite method

Run	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Temp (℃)	25	50	25	50	25	50	25	50	18.75	56.25	37.5	37.5	37.5	37.5	37.5	37.5	37.5	37.5	37.5	37.5
Time(hour)	2	2	5	5	2	2	5	5	3.5	3.5	1.25	5.75	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Solvent(×Powder)	10	10	10	10	30	30	30	30	20	20	20	20	5	35	20	20	20	20	20	20

3.Results and Discussion

3.1 Studying antioxidant power of extracts resulted from ethyl acetate

3.1.1 Studying antioxidant power with DPPH method

The results of the experiments showed that antioxidant power of extracts resulted from ethyl acetate increased with the increased temperature. As indicated in figure1, the amount of IC50 decreased with the increased temperature which showed the increased power of the antioxidant. As the temperature and solvent concentration increased, the antioxidant power of the resulting extract increased as well. However, in low concentrations of ethyl acetate and after a short time, the antioxidant power of the resulting extract decreased. The reason of this could be related to polymerization or the oxidation of existing phenolic compounds which were mentioned in Yilmaz's (2004) and Mansi's (2009) studies.

The increase of efficiency of extracting antioxidant compounds in line with the rise in temperature is probably due to improved mass

transfer and thus increased solubility. Moreover, the increased temperature of extraction can accelerate the diffusion of solvent and fortify the extraction of he impurities and wastes. Fan et al. in 2007 did an anthocyanin extraction from purple sweet potato which showed that there was a linear relationship between time and temperature of extraction with amount of anthocyanin, and as the time and the temperature increased, the amount of anthocyanin also increased.

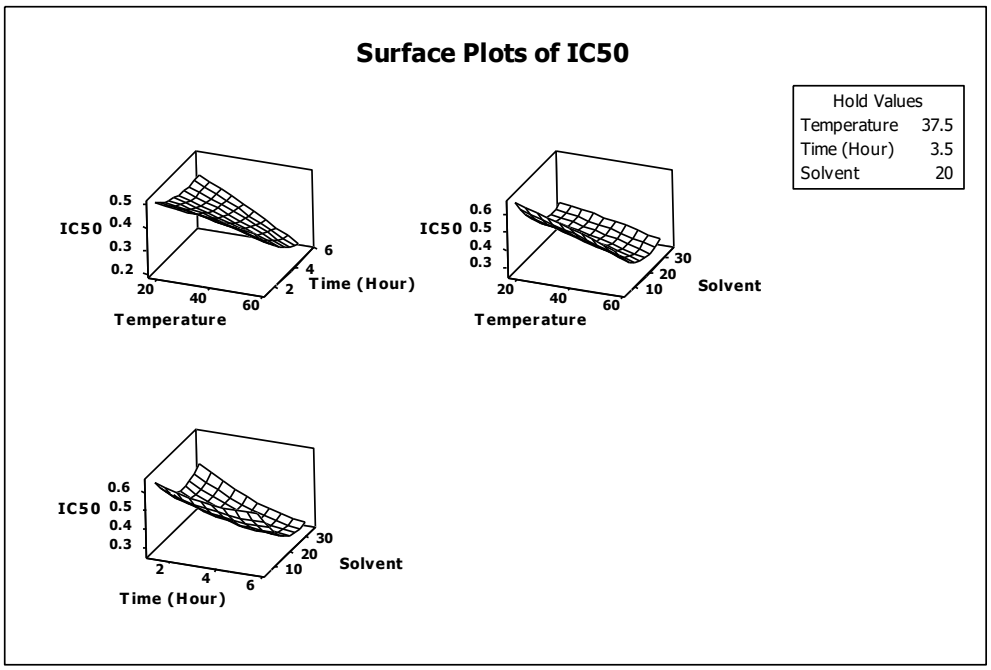

Figure 1. The effect of temperature, time and amount of ethyl acetate solvent on antioxidant power of the extract through DPPH method

3.1.2 Studying antioxidant power through FRAP method

The results showed that antioxidant power of achieved extracts from ethyl acetate through FRAP method rises with increased temperature, time, and amount of solvent. As mentioned before, the solvent diffusion coefficient increases with increasing temperature which leads to better exit of compounds. As indicated in figure2, in shorter time periods during extraction, increase of amount of solvent does not have much impact as longer time periods do on improving the antioxidant power of the extract. Gohari et al. (1394), in their study on antioxidant power of the extract resulted from interstitial wood of walnut fruit, reported that as time increased, the ferrous reduction power by the extract increased as well.

3.1.3 Studying antioxidant power based on the amount of total phenol

In this study, it was shown that at constant concentration of the solvent, an increase in time and temperature led to increased antioxidant power based on total phenol. However, in longer times, the intensity of antioxidant power was relatively reduced. The reason for such results could be that the impact of solvent could cause the extraction of impurities in longer contact time (Chan et al., 2009). In optimizing the extraction of phenolic compounds from wheat bran by using Ultrasound Method, wang et al. (2008) indicated that the amount of phenolic compounds from 10 to 30 minutes was significantly increased but was almost constant from 30 to 50 minutes. However, in fixed time and temperature, the increase of solvent concentration meaningfully improved

the power of antioxidant based on the amount of total phenol (acid Gallic).

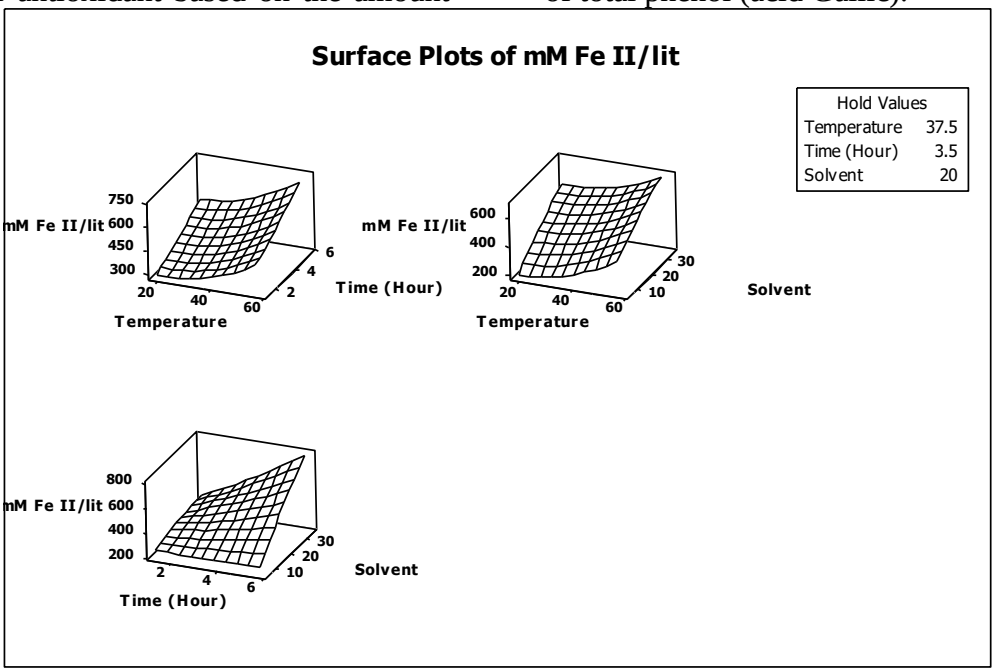

Figure 2. The effect of temperature, time and amount of ethyl acetate solvent on antioxidant power of the extract through frap method

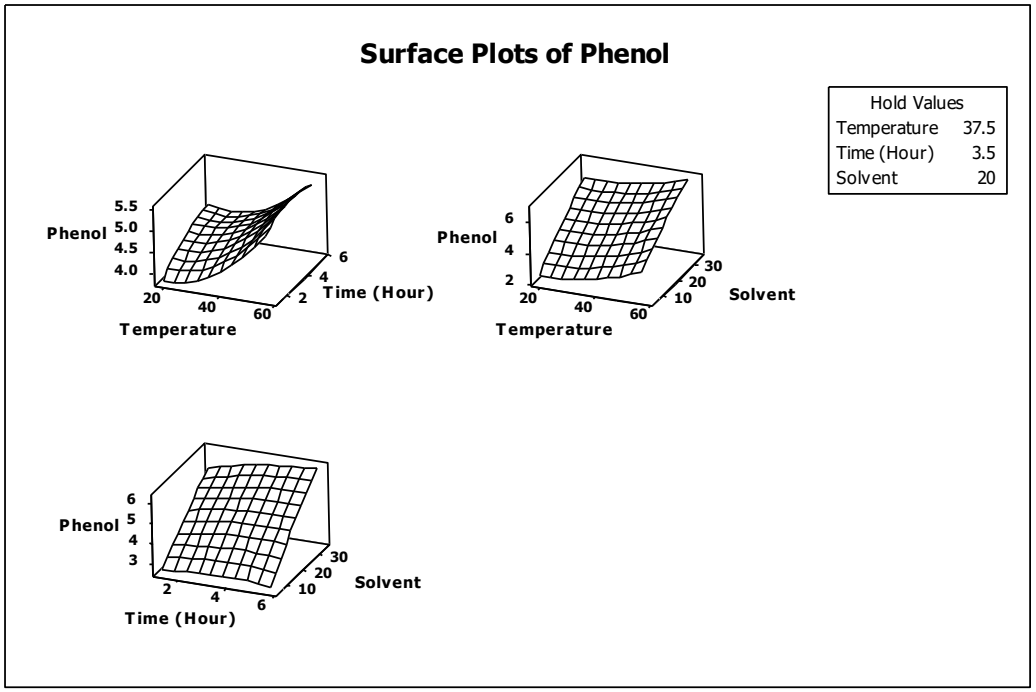

Figure 3. The effect of temperature, time and amount of ethyl acetate solvent on antioxidant power of the extract based on total phenol

3.1.4 Optimizing the best extraction conditions in terms of antioxidant power with ethyl acetate

Minitab software was used for optimizing the best conditions of extracting extracts with the highest power of antioxidant. For this purpose, first the importance of dependent variables was graded in Optimization Settings. Since FRAP test depends on time and pH intensity, and may not encompass the power of all antioxidant compounds, therefore this factor is considered less important than other factors (figure 4).

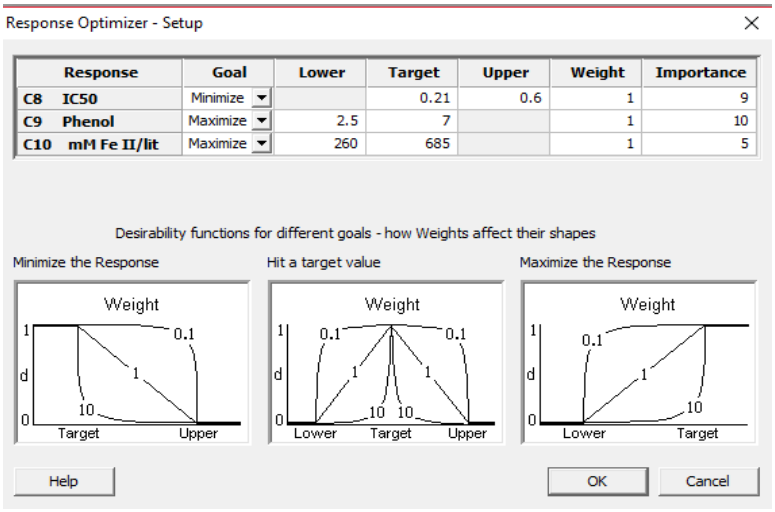

Figure 4. Trending in optimization process of parameters based on their importance in MniTab Software during extraction by ethyl acetate

According to the best extraction conditions, the best temperature of 56.25 ºC, time of 5.25 h, and solvent concentration of 35 times of quince peel powder with 99 % desirability was determined.

3.1.5 Determining the amount of Lutein extracted from the ethyl acetate extracts

The value of extracts was evaluated which were extracted by ethyl acetate based on the existence of healthy carotenoid of Lutein in two levels of temperature, time, solvent concentration regarding the remaining time of pure Lutein peak (2.243 min) and the area under its curve (Sheikhzadeh et al., 2015). Based on these and since the area under the peak of pure Lutein was 5000 and its concentration was 10 ppm, the concentration of other extracts could be computed (figure 5).

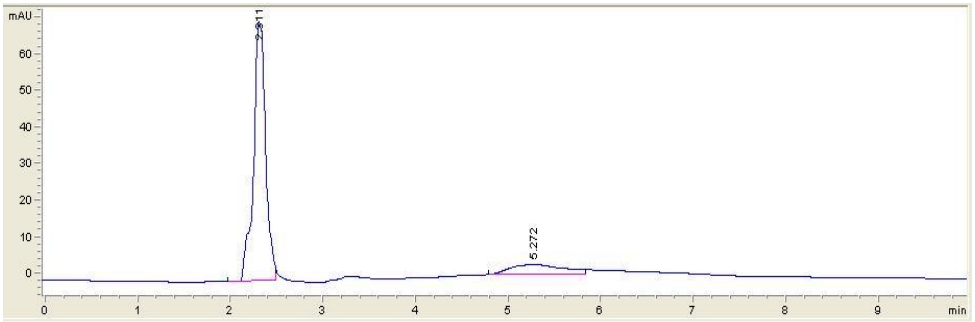

Figure 5. Standard chromatogram for pure Lutein (Sheykhzadeh et al., 2015**)**

Table 2. Comparing the amount of Lutein in different time, temperature, and concentration during extraction by ethyl acetate

Temperature (°C)	Time (hour)	Solvent concentration	Area under peak	Concentration (ppm)
25	2	10	407	0.814
25	2	30	895	1.79
25	5	10	682	1.364
25	5	30	1012	2.024
50	2	10	752	1.504
50	2	30	1965	3.93
50	5	10	1853	3.706
50	5	30	2021	4.041

Based on table 2, comparing to other extracts, the results showed that the amount of Lutein was more in the extract achieved under 50 °C for 5 hours and concentration of ethyl acetate 30 times of quince peel powder. Sheikhzadeh et al. (2015) also reported the highest amount of Lutein in carotenoid extract from banana peel in 40 °C.

3.2 Studying antioxidant power of extracts from hexane

3.2.1 Studying antioxidant power through DPPH method

According to figure 6, studying antioxidant power of extracts from hexane through DPPH method showed that in constant concentration of solvent, comparing to increased time, increased temperature was significantly more effective in improving antioxidant properties. Although in longer times, antioxidant power was relatively reduced.

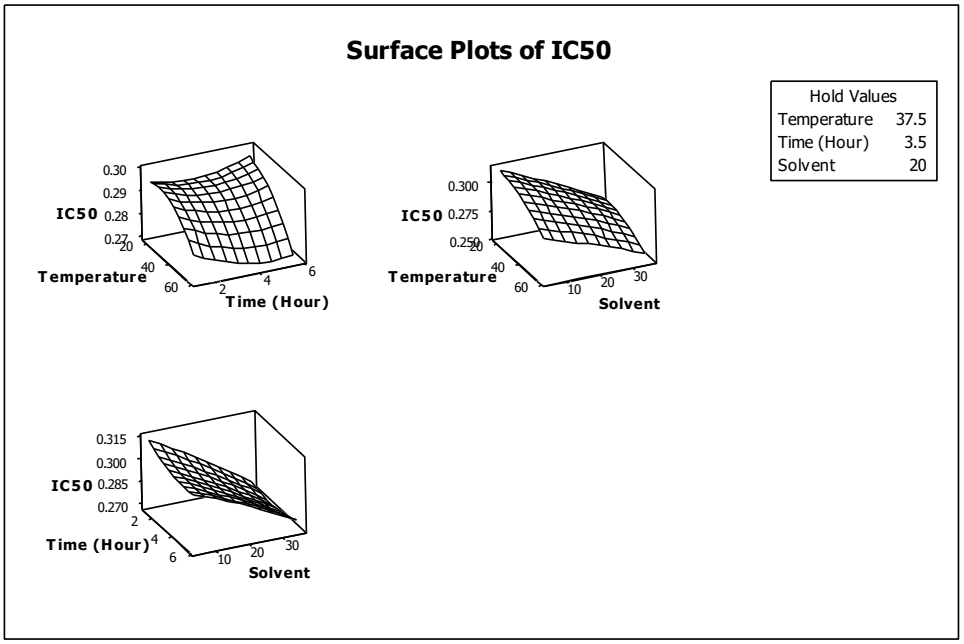

Figure 6. the effect of temperature, time and amount of hexane solvent on antioxidant power of the extract through DPPH method

But in all levels of temperature and time, increase in concentration of the solvent improved antioxidant properties.

Shaddel et al. (2001) claimed that the effect of time on DPPH radical-scavenging activity is significant and the amount of DPPH radical-scavenging activity is increased to a certain extent as the temperature increased (Lower IC 50). These researchers expressed that with increased extraction time, the scavenging activity increased to a certain extent.

Dong Rui et al. (2011) also studied the effect of time from 5 to 40 minutes on extracting cherry seeds and their results indicated that the radical-scavenging activity increased in the time of 5 to 30 minutes but then decreased. They also claimed that due to their decomposition, the temperature above 60 degrees led to a decrease in antioxidant power.

Kishk et al. (2010) said that DPPH radical-scavenging activity in ginger increased up to 30 minutes and then it became constant. Also the DPPH radical-scavenging activity of ginger increased from temperature of 20 to 54 degrees and then dropped because of the decomposition of antioxidant compounds.

3.2.2 Studying antioxidant power through FRAP method

As shown in figure 7, an increase in hexane concentration increased ferric reducing power which indicated an improvement in antioxidant power of extracts. However, constant concentration of solvent in higher temperatures and longer extraction time would reduce antioxidant power to some extent.

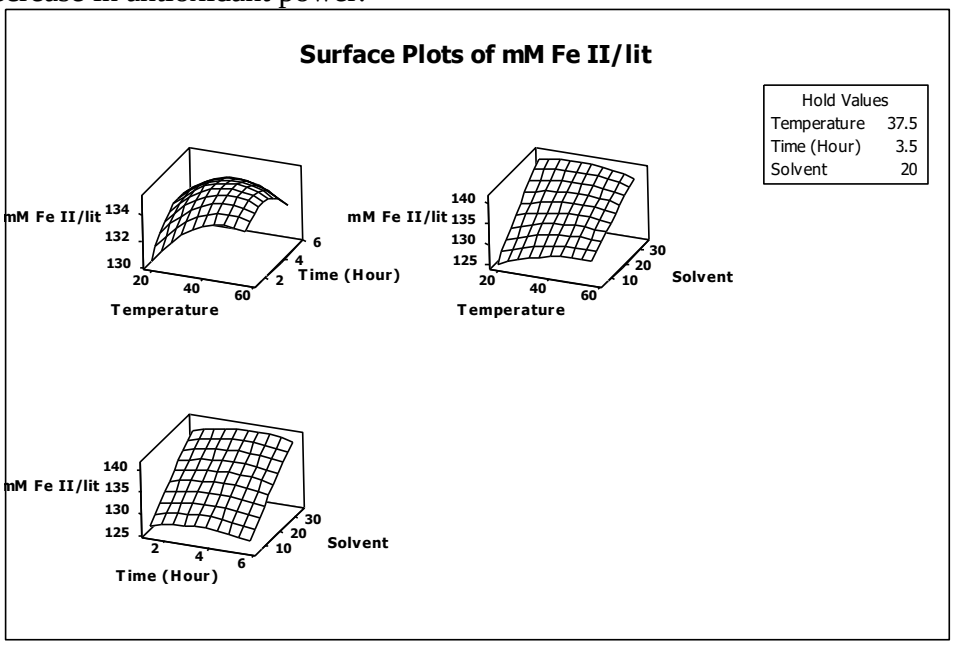

Figure 7. The effect of temperature, time and amount of hexane solvent on antioxidant power of the extract through FRAP method

The longer extraction times could possibly cause oxidation of polymerization of antioxidant compounds (Yilmaz, 2004; Mansi, 2009).

3.2.3 Studying antioxidant power based on the amount of total phenol

The results suggested that high concentrations of hexane in higher temperatures caused more extraction of phenolic compounds.

Although, longer extraction times did not have a significant effect on the amount of phenolic compounds in extracts. However, in higher temperatures, the amount of phenolic compounds decreased which could be due to thermal degradation of phenolic compounds (figure 8).

3.2.4 Optimizing the best extraction conditions in terms of antioxidant power with hexane

For optimizing the best conditions of extracting extracts with the highest power of antioxidant, Minitab software was used. Therefore, the importance of dependent variables was first graded in Optimization Settings. Since FRAP test depends on time and pH intensity, and may not encompass all power of all antioxidant compounds, therefore this factor was considered less important than other factors. And since hexane is a non-polar solvent and extracting its phenolic compounds can be less, therefore this factor was less important as well (figure 9).

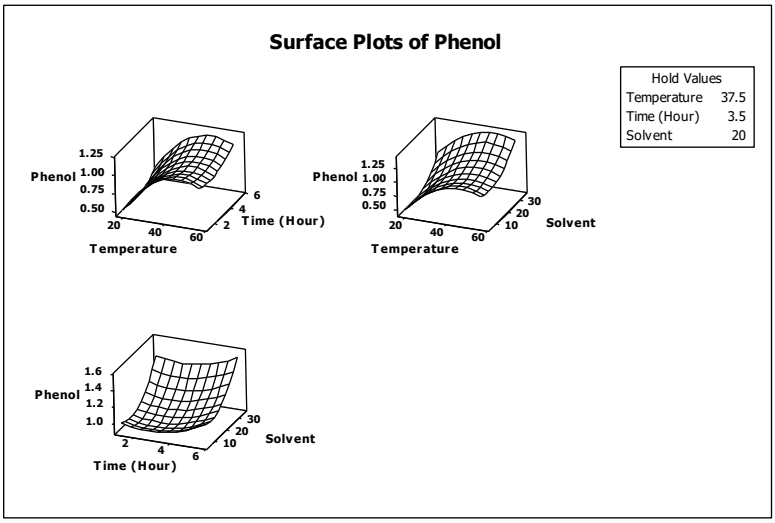

Figure 8. The effect of temperature, time and amount of hexane solvent on antioxidant power of the extract based on total phenol

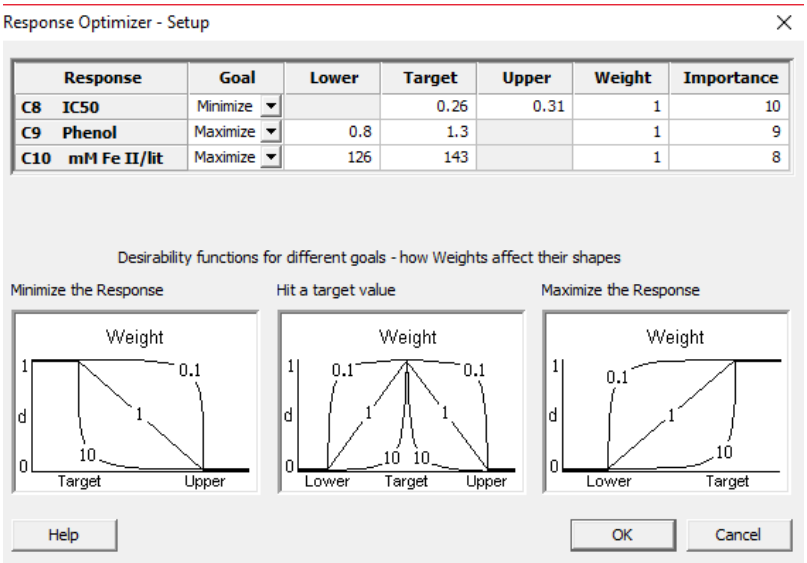

Figure 9. Trending in optimization process of parameters based on their importance in MniTab Software during extraction by hexane

According to this, the best condition for extracting hexane was determined in temperature of 50.56 ºC, time of 3.6136 h, and solvent concentration of 35 times of quince peel powder with 94% desirability.

3.2.5 Studying the amount of extraction of Lutein in extracts from hexane

The extracts from hexane were also evaluated based on the existence of Lutein in two levels of temperature, time, solvent concentration regarding the remaining time of pure Lutein peak (2.243 min) and the area under its curve (Khoshnevis et al., 2015). Based on the area under the peak of pure Lutein 5000 and its concentration of 10 ppm, the concentration of other extracts could be computed.

Table 3. Comparing the amount of Lutein in different time, temperature, and concentration during extraction by hexane

Temperature (°C)	Time (hour)	Solvent concentration	Area under peak	Concentration (ppm)
25	2	10	3195	6.39
25	2	30	3540	7.08
25	5	10	3201	6.402
25	5	30	3685	7.37
50	2	10	4120	8.24
50	2	30	4890	9.78
50	5	10	4136	8.272
50	5	30	4659	9.318

Based on table 3, the results showed that, comparing to other extracts, the amount of achieved Lutein was more in 50 °C, 2 h, and concentration of 30 times of hexane in proportion to the amount of quince peel powder. Sheikhzadeh et al. (2015) also reported the highest amount of Lutein in carotenoid extracts from banana peel in 40 °C. Khoshnevis et al. (2016) also considered 40 °C as the optimal temperature and methanol solvent as the best extract in terms of the amount of Lutein in the peel of Cucurbita pepo.

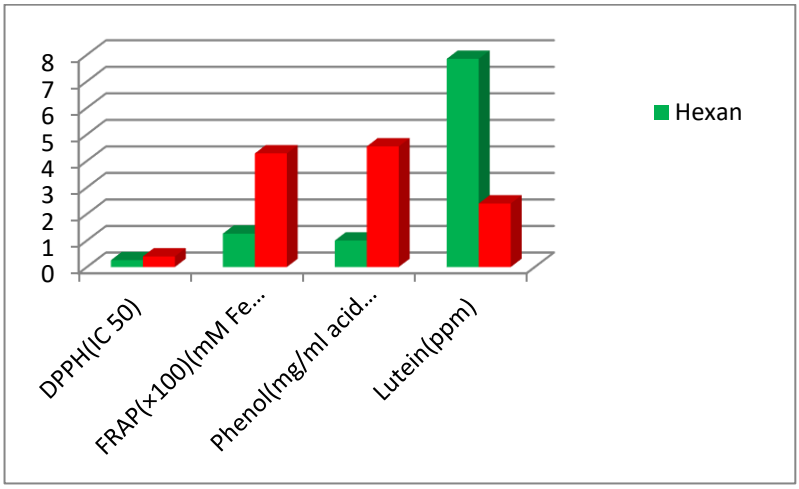

Figure 10. An overall comparison of the performance of two solvents of hexane and ethyl acetate in extracting antioxidant extract from quince fruit peel

3.3. A general comparison of hexane and ethyl acetate in antioxidant power of extracts

In figure 10, there is an overall comparison of two solvents in terms of their antioxidant power through three above mentioned methods.

As shown in the above figure, ethyl acetate is a polar solvent which could extract extracts with higher antioxidant power comparing to non-polar hexane. The reason for this could be due to presence of more polar antioxidant compounds in quince peel. However, hexane capacity as a healthy carotenoid is more than ethyl acetate in extracting Lutein. The reason for this was the Lutein hydrocarbon chain structure which gives it a nonpolar status, therefore, hexane performs better in its extraction as a non-polar solvent.

4.Conclusions

The present study investigates the effects of temperature, time, and two solvents on antioxidant power through FRAP, DPPH and total phenol methods and the amount of extraction of Lutein as healthy carotenoids. The results revealed that increased solvent concentration in all experiments led to improved extraction of antioxidant compounds and enhanced antioxidant power of the extracts. However, in high levels of temperature and time, the antioxidant power of the extracts has dropped which was due to the oxidation of carotenoids, phenol polymerization and maybe the extraction of impurities. The best extraction condition for extracting with ethyl acetate was 56.25 ºC, 5.25 h, and concentration of solvent 35 times of quince peel powder with desirability of 99% and for hexane solvent was 50.56 ºC, 3.6 h, and concentration of solvent 35 times of quince peel powder with desirability of 99% which was achieved by MiniTab Software. The most optimal amount of Lutein was achieved in extracting through ethyl acetate in 50ºC, 5 h, and solvent amount of 30 times of powder, therefore, the best amount of Lutein was determined in extracting with hexane in 50 ºC, 2 h, and solvent amount of 30 times of quince peel powder. Overall, the extraction of ethyl acetate was more successful in extracting antioxidant extract.

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INFLUENCE OF CLONE ADND ROSTOCK ON TOTAL POLYPHENOLS, CATECHIN, EPICATECHIN AND RESVERATROL IN RED WINE CABERNET-SAUVIGNON FROM SÎMBUREŞTI VINEYARD

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ABSTRACT

Six red wines obtained in the years 2012 and 2013 were analyzed in the Sâmbureşti vineyard from various clone / rootstock combinations, the Cabernet Sauvignon variety. All wines had high content of polyphenolic compounds, which is good for aging. Their sensory attributes in the first year after production were less appreciated at tasting, being very intense colored and abundant of blue pigments. Also, the smell was dominated by heavier, vegetal, grassy flavors that take time to improve.

1.Introduction

Wine is one of the most consumed beverages over the world containing large quantities of polyphenolic compounds. These compounds are responsible for quality of red wines, influencing the antioxidant activity, astringency, bitterness and colour, their composition in wine being influenced by the varieties, the vintage and the wineries (Hosu Anamaria et all., 2014). The quality of red wines is highly determined by the composition of phenolic compounds. Some of their sensory attributes, such as color, body and astringency, are mainly associated with the composition of anthocyanins and proanthocyanidins (Vidal S. et all., 2003). The red grape *Vitisvinífera* is an important source of phenolic compounds, which can prevent disease if included as a part of a diet. The levels of these compounds in grapes have been associated with various environmental factors, such as climate, soil composition, and biotic stress (Acuña-Avila P.E. et all., 2016). Grapevines are regularly subjected to environmental constraints, which are mainly soil water availability limitations and supra-optimal temperatures during summer. Even in cool to temperate climates, conditions of water stress may periodically arise during summer months, depending on vineyard topography and rainfall amounts. The pedoclimatic situation (soil type, soil available water reserves, rainfall) and climate evolution both play a major role in the development of edaphic water deficits. Global warming and heat waves, observed over the last few decades, have further accentuated abiotic stress (Zufferey V. et.all., 2017). Grape maturity at harvest is crucial and determines the final quality of wine. The maturity of grapevine berries is the combination of optimal technological and phenolic maturity (Rabot Amélie et.all., 2017). Grapes are qualitatively and quantitatively rich in phenolic compounds, which are secondary metabolites widely distributed in the plant kingdom. These phenolic compounds include

anthocyanins, flavonols and tannins, and are considered very important from a health point of view. Phenolics have received much attention in recent years because, in the human diet, they have been positively related to total antioxidant activity (Gil-Muñoz R. et.all., 2017). The concentration of these compounds in grapes depends on many factors, including the variety, growing conditions, climate, harvest year and winemaking techniques (Bavaresco L. et.all., 2012).

2.Materials and methods

2.1.Grape sample

Five Romanian *Vitis vinifera* grape clones of Cabernet Sauvignon variety (169/R140, 169/Gravesac, 169/R110, 337 and 685) from hilly areas of Oltenia - România were considered in this study. The variety clones, from the 2012 and 2013 vintage were grown in the same conditions in the vineyards Sâmbureşti.

2.2.Vineyard conditions and wine making

The grapes were collected in 2012 and 2013 from SâmbureştiVineyard.

The Samburesti vineyard is privileged by the strategic position it occupies in the transition zone between the getic hills and the long hills that make up the Piedmont of the Cotmeana. The Samburesti vineyard is situated in the southern part of the country at 44 ° 48 'north latitude and 24°48' south longitude, 30 km from the Dragasani vineyard and holds a privileged position due to its location in the transition zone between the getic hills and the long hills which make up the southern half of Piedmont, formed of the Southern Carpathians in the first part of the Quaternary. Climatic factors have a strong influence on the physiological and biochemical processes of the vine. Temperature influences the processes of growth and fructification both through its level and by the sum of temperature ranges within a defined time period. Along with humidity, it determines the area of botanical propagation and the economic culture of the vine. The sum of the annual temperature ranges, calculated over a period of 40 years, was about 3856 ° C, ranging from 3640-4295 ° C, and the average temperature was 15 ° C, a figure indicating the framing in the optimal viticulture areas.

The grapes were vinified in the Oenology Laboratory of the Faculty of Horticulture. In order to have the highest confidence in the results obtained and the safety of the minimization of the influence of external factors, it has been adapted as a working method the uniformization of all technological elements with an impact on the quality of the obtained wines. Thus, it was chosen to harvest all clones on the same day, to apply the same vinification technology sequences and wine tasting at regular intervals, plus repeating the schemes the following year. With the elimination of all the disturbing factors on the certainty of the results, there are two important elements that decisively emphasize the results: the potential of the clone-root combinations and the applied vinification scheme. Technological scheme used to obtain wines Cabernet Sauvignon was adopted by micro-vinification general scheme of red wine with grapes destemming.

2.3.Analytical method

All samples were analyzed using gas chromatography and following the method used by the Laboratory of the Department of Horticulture and Food science and the laboratory of National Institute for Cryogenics and Isotopic Technologies (I.C.S.I. Rm. Valcea).

2.4.Determination of phenolic compounds in wines. Folin-Ciocalteu method.

FCM based on the reduction of a phosphotungstate-phosphomolybdate complex by phenolic compounds to blue reaction products was used (Wildenradt& Singleton1974; Vinson et al. 1998; Singleton et al. 1999).

The total volume of the reaction mixture was mini-mised to 1 ml. Each sample (white wines 100 µl, red wines 50–100 µl diluted 10-fold) was read at 760 nm after 30 min of standing against blank (100 µl water instead of sample). Five-point calibration using 2mmol/l gallic acid as the standard was linear ($R^2 > 0.997$) up to the concentration of 0.2 mmol/l in the reaction mixture and the absorbance range up to 3.000 AU. The determined values were expressed as gallic acid equivalents (GAE). Highly repeatable results for standards and samples were obtained.

2.5.Statistical analysis

All parameters analized were assessed in triplicate, and the results were expressed as mean ± SD values of 3 observations. The mean values and standard deviation were calculated with the EXCEL program from Microsoft Office 2010 package.

3.Results and discussions

3.1. Results on the evolution of polyphenols wine composition

Determination of wine polyphenol composition obtained in 2012 and 2013, was performed by the analysis carried out in May, 2014 and October, 2015. The results of the wine polyphenols composition of the two years, both determinations are presented in Table 1.

The main parameter of the polyphenols composition is the total polyphenol content. The analysis of the data on the values of this parameter highlights that, overall, wines in 2012 have higher contents than those of 2013.

Thus, the wines of 2012, total polyphenol content is between 2216.6 and 2738.6 mg / L, while the wines from 2013, even if they are one year younger when determining content ranges from 1714.2 and 2726.3 mg / L.

In 2012, all Cabernet Sauvignon wines contain over 2200 mg / L total polyphenols, with differences between them up to 600 mg / L. For wines obtained in 2013, the contents are lower in all variants, although they are younger and normally during the evolution there is a general tendency to effectively reduce the total amount of phenolic compounds in wine as a result of precipitation phenomena. Under these circumstances, it would have been normal for younger wines to have higher contents in total polyphenols, but this has not happened. The explanation of this situation should be sought in the analysis of other factors influencing the polyphenols composition of wines, especially red ones. Thus, the most important factor influencing the polyphenols composition of the wines and, implicitly, the areas of spreading of the red wine variety culture are the specific climatic and soil conditions of the wine regions. In the present case, the pedological conditions were the same in both years of study, the experiences being located in the same place. That is why the factor that made the difference between the wines obtained during the two years of study in the polyphenols composition is the great difference between the wine years 2012 and 2013 regarding the climatic conditions. The abundance of phenolic compounds in grapes in 2012 certainly contributed to the fact that in that year the grape production was significantly lower than in 2013 due to the much lower precipitation regime in the maturing period.

In 2012, the largest total polyphenol content was in clone 169, primarily 169 / R140, and the lowest at clone 685, which is also true for wines from 2013. Not only the total polyphenol content varied greatly depending on the climatic conditions specific to the wine year, but also on the values of other phenolic compounds. Thus, the contents of catechin and epicatechin are also higher for wines in 2013 than for 2012 for all clones.

Of the other phenolic compounds that have been identified, the resveratrol content is of greater interest, given that this constituent is the one in which, in recent years, it is given a very important role in the beneficial influence of consumption red wine on health. The values of this constituent were, however, small and very small for both varieties in both wine years, regardless of the experimental variants studied.

Table 1. Polyphenols composition of wines in 2012

Variant	Total polifenols (mg/L)		Catechina mg/L		Epicatechina mg/L		Resveratrol mg/L	
	2 years	3 years	2 years	3 years	2 years	3 years	2 years	3 years
2012 Year								
169/R140	2715.5±100.0	2534.3±148.0	2.75±0.14	2.56±0.93	3.29±0.48	2,81±0.39	0,050±0.0004	0,040±0.001
169/ Gravesac	2623.7±94.50	2454.3±107.00	3.58±0.33	3.12±0.16	3.96±0.12	3,32±0.24	0,043±0.0008	0,032±0.001
169/R110	2738.6±30.00	2556,8±104.00	2,93±0.41	2.64±0.36	3,87±0.11	3,40±0.17	0,036±0.0008	0,022±0.003
337	2632.8±87.00	2412.3±108.50	2.38±0.33	1.88±0.40	2,93±0.14	2,44±0.34	0,056±0.0009	0,042±0.009
685	2216.6±83.00	1988.6±44.50	2.39±0.16	1.94±0.48	3,99±0.19	3,33±0.13	0,037±0.0011	0,024±0.009
Cabernet Sauvignon	2556.5±121.53	2212.2±100.15	2.90±0.04	2.45±0.26	3,45±0.22	2,88±0.21	0,044±0.001	0,030±0.014

Table 2. Polyphenols composition of wines in 2013

Variant	Total polifenols (mg/L)		Catechina mg/L		Epicatechina mg/L		Resveratrol mg/L	
	2 years	3 years	2 years	3 years	2 years	3 years	2 years	3 years
2013 Year								
169/R140	2726,3±88.15	2526,1±145.90	2,64±0.18	2,52±0.5	3,18±0.37	2,78±0.37	0,770±0.15	0,685±0.10
169/ Gravesac	2544,1±63.85	2116,3±93.25	3,48±0.53	3,02±0.35	3,81±0.10	3,22±0.33	0,568±0.16	0,502±0.09
169/R110	2698,4±29.15	2456,2±102.45	2,84±0.40	2,56±0.33	3,75±0.13	3,32±0,21	0,414±0.03	0,352±0.04
337	2618,3±89,15	2402,1±107.50	2,24±0,21	1,74±0.38	2,81±0.13	2,38±0.36	0,781±0.14	0,616±0.08
685	2195,2±80.00	1975,3±43.90	2,18±0,20	1,62±0.41	3,85±0.19	3,28±0.15	0,502±0.14	0,404±0.05
Cabernet Sauvignon	2515,3±119,55	2187,4±98.25	2,78±0.45	2,35±0.23	3,35±0.21	2,75±0.24	0,605±0.10	0,495±0.06

Regarding the influence of the viticultural year condition on the content of resveratrol, it has very low values for wines in 2012, from 0,037mg / L to 0,056mg / L at the first determination, and from 0,022mg / L to 0,042mg / L to next year's determination.

The large differences in resveratrol content between wines from the two years can be explained on the one hand by the difference in age between them, the content in resveratrol decreasing as the age of the wine grows. On the other hand, it is obvious that the wines of 2012 have content lower resveratrol climatic conditions specific year concerned, excessively dry, leading to the biosynthesis low resveratrol compared with a year wine with a more precipitous rainfall regime.

3.2. Results on evolution of organoleptic characters of wine

All wines, regardless of the year they were obtained, have been tasted 5 times. The results of the tastings clearly show a way of evolution typical of quality red wines, which are not suitable for consumption in the young stages of evolution, being unobtrusive to all types of features - visual, olfactory and gustatory.

3.2.1. Evolution of organoleptic characteristics in wines, harvest 2012

The organoleptic characteristics of wines obtained in 2012 are influenced to a considerable extent by the defining climatic characteristics of this wine year, which was one very special, very different from all the other wine years of the last decades. Thus, the beginning of 2012 was marked by a terrible winter, with large frosts and massive snowfalls, from the end of January and throughout February 2012 temperatures of -25^0C were recorded as they were not recorded in the vineyard of Sâmbureşti from the mid-80s of the last century.

From mid June until November no significant rain fell, which influenced the vegetative cycle of the vine, the phases of growth and maturation of grapes in these special climatic conditions, with successive and extreme meteorological phenomena, varieties which have suffered less from winter frost because of their resistance or location in the vineyard, have accumulated large proportions of sugar in grapes and have had an earlier baking compared to the regular years. Also due to the high temperatures and sunstroke in the second half of the summer and the onset of the autumn, combined with the lack of rainfall, the accumulation of large proportions of phenolic compounds in grapes was favored.

These natural elements have clearly influenced the quality of wines by their footprint on the quality of grape raw materials used in winemaking. Technological elements consisting of the 7-day maceration period, with the homogenization of the must phases, the partial decoction of the grapes, the use of pectolytic enzymes to stimulate the extraction and the levies selected for the control of the alcoholic fermentation imprinted the wines with the characters general coloring, aromatic and gustative intensity, body, structure, astringent, hardness. Differences in sensory perception and appreciation of sensory characteristics were determined by clones or their combinations with different rootstocks.

At the first tasting of wines, made almost in mid-December, all six wines was very intensely colored, almost black, with a very high density of red and violet pigments, which gave the colorless colors. The smells of all the wines were very intense and, overall, pleasant, the differences between them being very small, hardly perceptible even by the trained and competent tasters. In all variants there were quite pronounced vegetable odors (dominant notes being grasses, pepper and green walnuts), smells of berries, especially blueberries and pigeons, as well as mild fermenting CO_2 influences.

The taste test was the least agreeable part of tasting because of the harsh and very tangy taste of all wines, due to the high content of phenolic compounds (anthocyanins and tannins) with low polymerization rates. But also on the tasting exam were the differences between wines. If on the visual exam, all the

wines received the same ratings, the olfactory examination was the same, in the gustatory examination the differentiations resulted from the higher or lower contents in alcohol and glycerol, which decreased more or less of hardness, astringency or bitterness.

Under these circumstances, due to the dark, almost violet color, of the vegetable odors, and especially the harsh taste given by the hardness, the astringency and the toughness of the tannins, the scores obtained by the wines at the tasting were more than modest, being included between 72 points and 78 points. The smallest score was obtained from the wine obtained from clone 169 grafted on the R1010 rootstock, which definitely had the most harsh taste. The highest score (78 points) was obtained from clone 685 wine in combination with SO_4^{2-} rootstock, wine whose taste, although tough, showed more softness and a little more roundness.

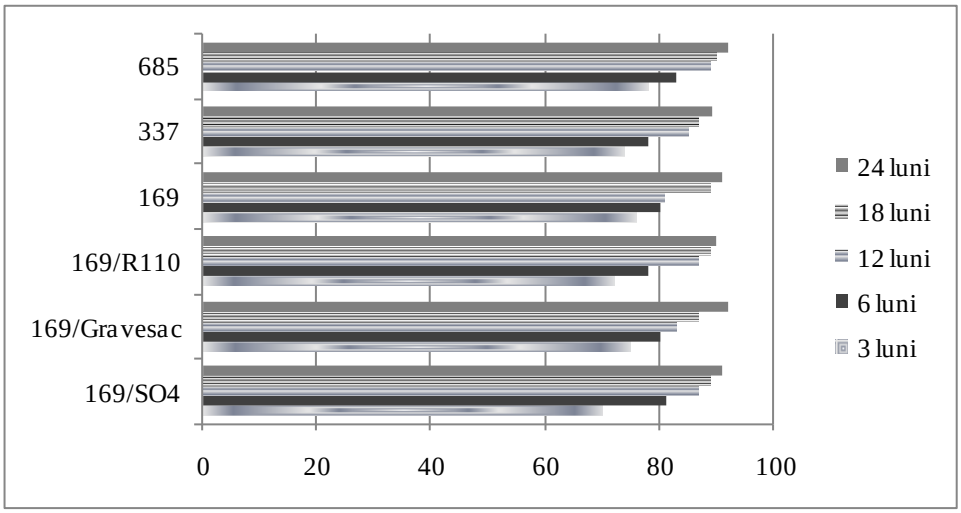

Figure 1. The wine tasting score of 2012

The second wine tasting took place during the next 3 months, at the age of 6 months of the wine, and on this occasion it was noted an improvement of sensory perception of wines, materialized also in bigger tasting, so all 6 wines obtained 4 or 5 points more than the 3-month tasting. It is very important to note, with regard to the characteristics examined at the tasting, that this time all 6 wines were evaluated with the same ratings in terms of clarity and appearance.

The next tasting took place in 6 months, so when the wine was 1 year old from the vinification.

On this occasion, it was observed in all wines the significant increase of wine tastings by the tasters, naturally due to the improvement of the organoleptic characteristics of the wines due to the phenomena that took place in the past interval from the previous tasting phenomena which consisted in the first in combinations,recombination's, polymerizations and condensations between tannin and anthocyanin molecules, led to the reduction of astringency and tannin hardness, accompanied by an improvement in roundness and velvety.

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accompanied by an improvement in roundness and velvety.

The next tasting took place after another 6 months, 1 year and a half after getting it. On this occasion there was a continuation of the improvement of wine appreciation and, implicitly, of the scores given by the tasters, but compared to the previous tasting, which was also carried out 6 months after the other, the rhythm of the score increase was obviously smaller.

The smallest increase was 1 point, at 685 / SO4 (from 89 points to 90 points), but it still remained after this 4th tasting. The most important point increases were 3 points, recorded on 3 wines: 169 / R110 (from 84 points to 87 points), 169 / Gravesac (from 87 points to 90 points) and cloned wine 169, combination between clone / rootstock variants (from 86 points to 89 points).

Wine of clone 337 / Gravesac increased from 85 points to 87 points and remained in last place after this tasting tie wine from clone 169 / R110.At the last tasting, the two year old, continued the process of improving the organoleptic characteristics of the wines, materialized in the increase of the awarded scores. These increases were very close between wines, 1-2 points, so the order of the scores was kept. Making a comparison between wine tastings at the first tasting, from the age of 3 months and the tasting at the age of 2 years, it is noted that during this interval there were significant improvements in how the wines and the scores attributed to the tasting. If at the 3 months tasting the wines were rated with scores ranging from 72 points to 78 points, at the 2-year tasting the scores were between 89 points and 91 points, which means good for very good. During this time, almost all the organoleptic characteristics of wines have improved as a result of the changes that took place in wine during their evolution and the age of 2 years is not yet an age where wine can be said to have evolved sufficiently for to be consumed. Therefore, going through maturing in an oak wood barrel, followed by aging in glass, is a mandatory step through which these wines must pass before reaching the table of consumers.

3.2.2. Evolution of organoleptic characteristics in wines, harvest 2013

The wines obtained in 2013 were tasted at the same time interval as those of the previous year. Based on the results of these tastings, it can be noted that there are important similarities in terms of the tasters' expression and appreciation of the organoleptic characteristics of these wines between 2012 and 2013 wines, although there are some differences as to how they were perceived and evaluated the different clone-root combinations. First of all, it should be noted that in 2013 it was quieter in terms of climatic conditions than in 2012. Thus, there were no frost phenomena or snow comparable to the previous year, which means winter it was milder and did not cause any damage. The summer was not as droughty as in the previous year, there were also periods of 2-3 weeks without rain, but there were 3-4 heavy rainfall during the summer and it was important to say, all rains fell during the night, which means that although there were precipitations, they did not affect the duration of sunshine. Such a situation is particularly favorable and is particularly favorable and desirable for viticulturists and winemakers because it allows both the growth of grape grapes, better than the dry years, thus with higher yields and their maturing in the world, especially to red wine wines that can accumulate more anthocyanins in long-lasting sunshine skins. On the whole, the 2013 wine year was a very good year for both production and quality, such a situation being less common, as there is usually an inversely proportionate relationship between productivity and quality. At the first tasting of wines in 2013, the scores ranged between 70 points and 77 points, thus a little lower than in 2012, because most of the wines were somewhat more tanned, astringent, harder to suspect at this cruel stage their evolution. An important difference from the previous year was that wine obtained from Clone 169 / R110, which

received the lowest score in 2012 at the first tasting in 2013, received the second score, so it was better in 2013 than in 2012 Instead, the best-graded wine was, in 2012 and 2013, all wine from clone 685 / SO4, with the same score, practically (78 points in 2012 and 77 points in 2013).At the 6-month tasting, as in the previous year, wines improved some of their characteristics (the same as in the previous year), being better appreciated and getting higher ratings. The wine with the lowest increase in the tasting score in the 3 to 6 month period was the one obtained from the combination of combinations of clone 169 at which the increase was 5 points from 75 points to 80 points, which however meant that at the 6-month tasting it was ranked 3 in an indicative ranking of the scores, as well as at 3 months.

At the 1-year tasting, the wine from clone 685 had the highest scoring increase, reaching 87 points and consoling as the best-graded organoleptic wine, primarily due to its visual and olfactory features. Wine from clone 169 / SO4 have grown by 3 points, as well as the other wines of the same clone, reaching 84 points. The least raised the score of wine in clone 337 (2 points), remaining on the last place, with 81 points. The most important increase in fruit scores over wine ranges from 12 to 18 months, meaning a significant improvement in color, smell and taste in all wines.

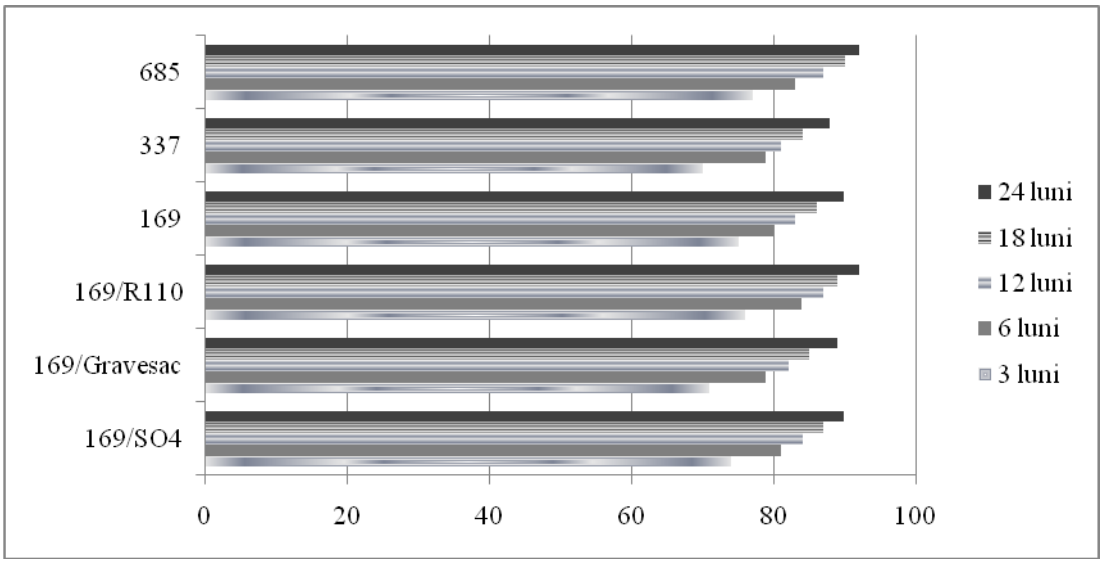

Figure 2. The wine tasting score of 2013

4. Conclusions

All 6 Cabernet Sauvignon wines showed in both 2012 and 2013 that their phenolic composition and organoleptic characteristics are very good wines with great potential for evolution and are intended for consumption as mature and outdated wines. The conditions in which they were developed, with 7-9 day maceration periods, with pectolytic enzymes, selected yeasts, fermentation activators led to the obtaining of organoleptic properties that do not allow their being put into use as young wines but only after maturation and aging.

Even if at the first tasting, for three months in both winemaking campaigns, the wines had organoleptic properties that make them difficult to consume, this means lack of quality, potential for evolution or freshness. It was very important that the wines did not present unpleasant odor or taste characteristics due to technological accidents, illnesses, odors or foreign tastes. Wines were less well appreciated for tasting on the background of colorless features without visual pleasure, being very intense colored and with an abundance of purple pigments, very unpleasant in appearance. This is not a color problem but is

normal for wines with only a few months, obtained from varieties with a high potential to accumulate pigments in the skin and by technological processes favoring the extraction of phenolic compounds from the solid parts of the grapes, respectively skins and seeds. Wines were not well appreciated or smelled. Even if in terms of the intensity of the flavors they presented well and very well, there were no revealed final pleasant flavors, the aromatic background being dominated by heavier odors, vegetal, grassy, weedy, with certain influences given by the presence of traces of CO_2 released from fermentative processes through which wines have passed. Even these olfactory features, even if they are not very pleasant, should not be considered defective, being explicable at this stage of evolution and subjected to changes in the later stages of wine evolution. The taste of all the wines was very poor, hard to accept, hard, harsh, lacking in fines, but it is also normal at this age. In fact, these unpleasant organoleptic characteristics are those that make wine not for consumption at this stage. The organoleptic characteristics of all wines improved significantly from one tasting to another, as they evolved. Continuous improvement of organoleptic attributes during 2 years of evolution shows that they are not fast-growing wines. This favorable evolution on the organoleptic plane is closely related to the evolution of the polyphenols complex, which was characterized by the decrease in the total polyphenols, catechin, epicatechin and resveratrol, as a result of the phenomena of condensation and polymerization.

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POSSIBILITY OF ANIMAL PROTEIN APPLICATION IN SAUSAGE PRODUCTION

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ABSTRACT
The article presents the results of the study undertaken to determine the effect that a protein-fat emulsion based on ScanPro T95 animal protein solution has on the physical, chemical and organoleptic parameters of sausage products. There has been developed the technology and formula for the production of cooked sausages in which meat raw materials were partially replaced with a protein-fat emulsion. The sausage food value has been calculated. The study of quality indicators has been carried out. The organoleptic parameters of sausage products with a partial replacement of meat raw materials with a protein-fat emulsion have been evaluated.

1.Introduction

Given the shortage of meat raw material and the desire of producers to reduce the production cost, alternative sources of protein and its derivatives are extremely relevant for the domestic meat market. That is why vegetable and animal proteins are widely used today in the production of meat products (Arihara, 2006). Such proteins allow an equivalent replacement of the missing expensive meat raw material.

Additionally introduced proteins have a positive stabilizing effect on the meat system (Gnanasambandam, et al., 1992). Meat products are supposed to possess certain consumer properties, such as juiciness, tenderness, certain degree of bite, density, etc.

Both vegetable and animal proteins contain essential amino acids but their amount is different. For example, blood plasma protein is considered to be complete because it contains all essential amino acids.

Many manufacturers use a pork skin emulsion of their own production. But this emulsion can't be an alternative to the industrial proteins (Heinz, et al., 2007). Emulsions manufactured independently have a number of shortcomings. Only young animals should be skinned, the skin should be carefully degreased, have no bristle, bruises or sores. It is not always the case, which poses the risk of microbiological contamination of the emulsion and as a result of the final product. Production of such emulsions requires preliminary preparation, additional time and labour. The grinding process leads to the wear of the working parts of grinding machines. Disruption of the raw material supply or decrease in its quality affects the manufacturing process and requires the process adjustment. Moreover, the prepared emulsion cannot be added to the prine for injection (Almeida, et al., 2014).

Proteins of animal origin in the form of dry powder is a relatively new type of food additive. Animal proteins were used in the production of meat products for the first time in the late 1980s. Nowadays, a wide range of animal proteins developed mainly by European companies is successfully introduced (Zorba, et al., 1995).

Pork skin proteins and beef proteins (produced from collagen-containing raw material) are in high demand among the producers. Bovine blood, raw meat and whey serve as raw material for the animal proteins production as well (Akoh, 1998).

The classification of animal proteins is similar to that of vegetable proteins; it is based on the content of the mass fraction of the dry protein component. However, only vegetable proteins are generally used in production of concentrates and isolates. The protein content in isolates can reach 100% (Akesowan, 2008).

The great interest in animal proteins on the part of technologists is explained by their unique properties. Combining meat and animal proteins provides great opportunities for producing quality food (Marianski, et al., 2010).

Animal proteins are usually 100% natural product, and their production is based solely on thermal (degreasing, dehydration) and mechanical processes (grinding). Complete animal proteins significantly exceed vegetable ones in terms of biological value. It has been proved that animal proteins compared to vegetable ones have a better balance of amino acids and meet the needs of human organism in the essential amino acids.

As a rule, functional animal proteins have neutral smell and taste, which is an obvious advantage over soy proteins. There is no need to use taste modifiers to neutralize the characteristic bean odor caused by the oxidation of lipids under the influence of lipoxygenases (Delgado-Pando, et. al., 2010).

Animal proteins significantly improve the rheological properties of food products, especially their consistency, at the same time performing stabilizing, gelling, and gelatinizing function, as well as improving the appearance of finished products (Xiong, 2012).

The reasons for the growing interest in functional animal proteins are not limited to their biological value, rheological and organoleptic properties. The significant advantages of animal proteins include their multipurposeness, ease of use, preservation of their properties during long-term storage in dry rooms without any special temperature conditions (up to one year), as well as potential yield enhancement and high profitability of production through their use (Feng, et. al., 2002).

The nutritional value of animal protein is much higher than that of vegetable protein. It is also known that the digestibility of animal proteins is 78-90%, whereas that of plant proteins is only 54-75%.

Proteins of animal origin have a higher nutritional value than vegetable proteins (Ahmad, et. al., 2010).

Connective tissue proteins, including collagens, are now classified as analogues of dietary fibers judging by their physiological effect, i.e. cytoprotective action and normalizing effect they produce on the intestinal microbiocenosis. They prevent dystrophic changes in the epithelium and the permeability of the cell barrier of the colon mucosa, which reduces the penetration of bacteria, toxins and various polymeric residues capable of causing allergy. It has been found that collagen proteins improve intestinal motility, and the products of its disintegration formed during heat treatment stimulate the secretory function of the organism, which increases the biological value of the product due to the maximum absorption of the components (Pasupuleki, et al., 2010).

Whey protein additives enrich meat products with complete proteins (albumins and globulins), increase viscosity and emulsifying ability; improve taste and tenderness of finished products.

The high nutritional value of meat-producing animals' blood is explained by a significant content of proteins, mineral salts, enzymes, vitamins, sugar, lecithin and other substances. The protein content of blood is practically the same as that of meat and contains only 5-10% more water (Akesowan, 2013). Whole blood and its fractions contain the majority of amino acids, and the number of essential amino acids significantly surpasses meat. In addition to nutritional and biological value, blood proteins have good functional properties, which is especially important in

sausage production (Yetmin, et. al., 2001). The use of edible whole blood in the production of meat products has some limitations due to its dark color, so the most acceptable way of using proteins obtained from the blood of meat-producing animals involves its complex processing.

High functional properties of animal proteins are manifested in their water holding capacity.

The water holding capacity of Supergel animal protein (Germany) is almost 4 times greater than the similar properties of Supro 500 E isolate, almost 10 times greater than the soy flour and soy concentrate, and the water binding capacity of individual proteins ranges from 400 to 700% (Peng, et. al., 2009).

The gel-forming capability of denatured protein preparations used in the meat processing industry directly affects the quality of the finished product. Functional animal proteins such as Supergel, ScanPro E-95, Atari ST 97 and several others are capable of forming a strong gel and thereby fixing the structure of the finished product without special heat treatment at a denatured protein concentration of 5% and higher in the "protein-water" system. Effective gels for sausage production can be obtained by hydration, for example, of one part of the Supergel protein and 15 parts of water at room temperature (Smith, et. al., 1973).

The water holding properties of animal and soybean proteins sharply increase with heat treatment above the denaturation temperature of the main protein components. When exposed to heat, the collagen in animal proteins "folds" as a result of the breaking of hydrogen bonds in the peptide chains. The change in their mutual position in the structure of the tropocollagen is accompanied by its loosening, increased hydration and an increase in the availability of peptide bonds to the action of proteases. Similar process occurs during preparation of protein-fat emulsions, as well as emulsions using functional animal proteins, fat and pig skin. At the same time, the protein value of the products, as studies have shown, only slightly depends on the heat treatment of the gel or emulsion, if the water temperature does not exceed 95° C.

Practice shows that the greatest efficiency of functional animal proteins is achieved through their preliminary hydration, preparation of a protein-fat emulsion, as well as emulsions with pig skin using hot water (Pasupuleki, et. al., 2010).

Protein-fat emulsions allow rational use of fatty raw materials (pork or beef raw tallow, fat trimmings, mid-back fat, cheek trimmings, flank, boiled or raw pork skin), introducing it into sausage forcemeat in a stabilized form (Huicho, et. al., 2013).

Given the versatility of the functional, technological and rheological properties of animal proteins, they can be used in combination with vegetable, which, on the one hand, reduces the cost of manufactured products and on the other, increases the nutritional value of the product, improves its taste and appearance (Meltem, 2006).

2. Materials and methods

2.1. Materials

Scanpro T95 animal collagen based protein solution (manufactured by Dansexport, Denmark) consisting of natural collagen, vitally important for the human body, was selected as an object of the study.

The protein-fat emulsion was introduced into the formula of the model forcemeat system at the stage of making forcemeat on the cutter. The forcemeat with no added protein-fat emulsion was used for control. Control and test samples of forcemeat systems were prepared according to a standard formula for sausage products.

2.2. Methods

The mass fraction of moisture was determined by drying the sample weight with sand to constant mass at a temperature (103 ± 2) ° C.

The mass fraction of protein was determined by a Kjeldahl-based method of the sample's mineralization and photometric measurement of the indophenol blue colour intensity, which is

proportional to the amount of ammonia in the mineralase.

The mass fraction of fat was determined by the extraction method using the Soxhlet extractor.

The mass fraction of ash was determined by drying, charring, and ashing at a temperature (550 ± 25) ° C of the test sample and subsequent calculation of the total ash mass fraction.

The pH was determined using a potentiometer (pH-150M).

Determination of water binding capacity of meat (WBC). The employed method of centrifugation is based on the separation of the sample's liquid phase under the action of the centrifugal force, the sample being in a fixed position. The amount of the liquid phase depends on the degree of interaction of moisture with the "frame phase" of the sample.

3.Results and discussions

The study of the general chemical composition of ScanPro T95 additive showed that the mass fraction of protein in the additive is 88.2%, fat 7.2%, moisture 3.2%, and ash 0.71% (Table 1).

Table 1. ScanPro T95 Chemical Composition

Additive	**Mass fraction, %**				
	water	**protein**	**fat**	**ash**	**carbohydrate**
ScanPro T95	3.20±0.06	88.20±2.87	7.20±0.21	0.71±0.01	0.69±0.02

As can be seen from the table, Scanpro T95 is rich in protein, which, when making sausages, enhances the functional and technological properties of the meat system.

In the course of the study, the technology and formula for cooked sausage production using ScanPro T95 was developed. The formula of the protein-fat emulsion for cooked sausages is given in Table 2.

Table 2. Formula of Protein-Fat Emulsion for Cooked Sausages

Ingredients	**Weight, kg**
ScanPro T95	3.22
Fatty raw material	48.44
Water	48.44
Total	100

The fatty raw material loaded into the cutter, pre-ground in the meat mincing machine with a hole diameter of 2-3 mm, was processed to a paste-like state. Then ScanPro T95 was added and thoroughly mixed with fat for 1-2 minutes in the cutter. Then cold water (18-20° C) was added and the whole mixture was processed in the cutter at the maximum speed until the emulsion was formed (Chang, et. al., 1997). The emulsion was poured into prepared containers in layers of not more than 20 cm in height and cooled at a temperature of 0-4° C. After cooling, the emulsion is supposed to have a dense consistency.

Control and experimental samples of forcemeat systems were prepared according to the formula presented in Table 3.

Determination of water holding capacity (WHC). The employed method is based on evaporation of moisture from the test sample with a special device, and calculation of the remaining amount of moisture in the test sample according to the formula.

Determination of fat holding capacity (FHC). The employed method involves using a refractometer to determine the refractive index of the test sample, and calculating the FHC value of the sample according to the formula, where the key index is the refractive index.

Determination of emulsifying capacity (EC) and emulsion stability (ES). To determine the emulsifying capacity, a homogenized mixture of the test sample and refined sunflower oil is centrifuged. After this, EC is calculated according to the

formula. The ES is determined by its heating, cooling and further centrifugation. After this, the volume of the emulsified layer is determined and the ES is calculated according to the formula (Skurikhin, et. al., 1998).

All measurements were carried out in three replications. Statistical analysis was performed using Microsoft Excel XP and Statistica 8.0 software package. The statistical error of the data did not exceed 5% (at 95% confidence level).

Table 3. Formula for Control and Test Sample of Forcemeat Systems

Ingredients	Test Sample	Sample 1	Sample 2	Sample 3
Unsalted Raw Material, kg per 100 kg				
Trimmed beef, grade 1	30	30	30	30
Trimmed semi-fat pork	49	44	39	29
Fat pork or sausage fat	20	20	20	20
ScanPro T95 based protein-fat emulsion	-	5	10	20
Dry whole cow milk	1	1	1	1
Total	100	100	100	100
Spices and additives, g per 100 kg of unsalted raw material				
Food salt	1900	1900	1900	1900
Sodium nitrite	7.5	7.5	7.5	7.5
"Milk" complex flavor-aromatic additive	950	950	950	950
Active Red dye	130	130	130	130
Fresh garlic	300	300	300	300
Emulsifier	500	500	500	500

Among physical and chemical parameters, pH, fat and protein content were calculated. The results are given in Table 4.

When ScanPro T95 was used as a part of the protein-fat emulsion, the moisture content of the model forcemeat system decreased to 56.62% simultaneously reducing the protein content to 11.19% and increasing the fat to 21.99% versus 21.86% in the control sample.

Since acidity affects the state of proteins in the meat system and their ability to bind moisture, a change in pH in the forcemeat injected with the protein preparation was studied.

The pH of the forcemeat with no protein-fat emulsion, which we accepted for control, was 5.9. The addition of protein-fat emulsion to the forcemeat resulted in an insignificant increase in the pH value to 6.02, which suggests an increase in the charge of forcemeat proteins and an increase in strength in the "water-protein" system. The change in the moisture content of the forcemeat was studied as well. The moisture content in the beef forcemeat control sample was 56.88%. When introducing the protein-fat emulsion with various proteins, the moisture content decreased (Hollingworth, 2010). The introduction of the protein-fat emulsion helped to reduce the moisture content in the forcemeat by approximately 5%, which in turn contributed to the increase in its fat holding capacity (FHC).

Stability of the forcemeat is a more complex generalizing indicator. It characterizes the development of the water binding capacity of the raw forcemeat, as well as the water- and fat- holding capacity of

the forcemeat, subjected to heat treatment (Jalal, et. al., 2014).

Functional and technological parameters of the model forcemeat before heat treatment are given in Table 5.

Table 4. Physical and chemical parameters of control and test samples of forcemeat systems

Physical and chemical parameters	**Test Sample**	**Sample 1**	**Sample 2**	**Sample 3**
pH value	5.91	5.94	5.98	6.02
Mass fraction, %				
protein	13.26	12.55	12.09	11.19
fat	21.86	21.93	21.96	21.99
moisture	56.88	56.11	55.43	54.03

Table 5. Functional and technological characteristics of model forcemeat before heat treatment

Indicators	**Model meat system**		
	Control	**5 % replacement**	**10 % replacement**
WBC, %	79.78±1.14	80.22±0.24	81.59±0.12
WHC, %	90.11±0.01	91.33±0.54	91.94±0.18
FHC, %	66.56±0.42	71.33±0.76	72.27±0.81
EC, %	71.47±0.52	73.35±0.19	75.98±0.79

When studying the functional and technological properties of the protein additive, it was found that the water binding capacity of ScanPro T95 was 10.9 mg of water per 1 g of the product.

The study of the fat holding capacity of the protein additive revealed that it is able to bind a fairly large amount of fat. The fat holding capacity of ScanPro T95 was 2.7 g fat per 1 g of the product.

The results of the study showed that the highest emulsifying capacity of ScanPro T95 is observed at a protein:fat:water ratio of 1:20:20. It was also established that the stability of emulsions after heat treatment decreases with a decrease in protein concentration in emulsions stabilized with ScanPro T95 connective-tissue based protein solution.

Connective-tissue proteins produce strong gels, which however are unstable with the rise of temperature, which can affect the quality of the finished product. Therefore, ScanPro T95 was introduced into the formula in form of a protein-fat emulsion.

After the forcemeat was ready, it was stuffed into casings. Then sausages were hung and later subjected to heat treatment by roasting or boiling, followed by cooling under a cold shower.

The finished product was tested for organoleptic properties – one of the most important indicators of nutritional value. The characteristics of the indicators given in Table 6 were accepted as the standard.

The organoleptic evaluation of the samples is shown in Fig. 1.

Table 6. Quality indicators of cooked sausage

Indicators	**Characteristics**
Appearance	Sausages in casing, dry surface, no unsmoked spots in the casing, no fat or water pockets
Consistency	Elastic
Colour and appearance on the cut	Pink or light pink, the forcemeat is evenly mixed
Smell	Characteristic of such products, no off-odor, with the aroma of spices
Taste	Characteristic of such products, no off-odor, moderately salty

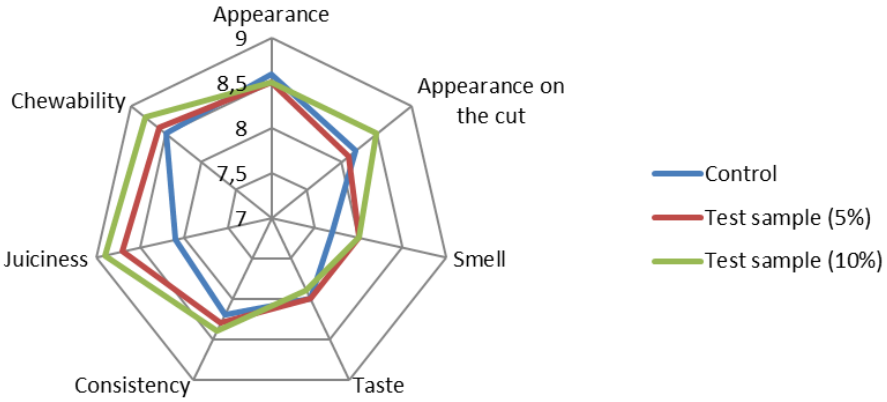

Figure 1. Organoleptic Evaluation of Sausage

Based on the study of the quality level, it was found that the protein-fat emulsion in an amount of 5% and 10% (sample 1 and sample 2) did not impair the organoleptic characteristics of the finished product. The samples had a pleasant taste, appearance and colour on the cut; tender and juicy consistency. The product's consistency remained monolithic, while the juiciness and tenderness increased. Adding 20% of the protein-fat emulsion to the forcemeat resulted in excessive softening of the consistency, appearance of fat pockets and a noticeable decrease in the meat taste of the products.

Thus, it appears optimal to replace 5% and 10% of meat raw material with the protein-fat emulsion.

The energy value of the finished product is given in Table 7.

Table 7. Energy value of the finished product

Indicators	**Control sample**	**Sample 1**	**Sample 2**
Protein, g	12.89	12.50	12.07
Fat, g	22.02	21.90	21.98
Carbohydrate, g	0.89	0.87	0.88
Energy value, kcal	253.3	250.58	249.62

The table suggests that the introduction of a larger amount of the protein-fat emulsion reduces the energy value of the finished product.

4. Conclusions

Based on the results of the research conducted, the technology of manufacturing cooked sausage with the use of the stabilized ScanPro T95 connective-tissue based protein solution has been developed.

There has been developed a formula of a protein-fat emulsion for cooked sausages. The physical and chemical properties of forcemeat systems have been studied. It has been proved that the use of ScanPro T95 as a part of the protein-fat emulsion decreases the moisture content of the model forcemeat system to 56.62% while reducing the protein content to 11.19% and increasing the fat to 21.99% versus 21.86% % in the control sample.

When studying the functional and technological properties of the protein additive, it has been found that the water binding capacity of ScanPro T95 is 10.9 mg of water per 1 g of the product.

Based on the study of the quality level, it has been found that introducing 5% and 10% of the protein-fat emulsion (sample 1 and sample 2) does not impair the organoleptic characteristics of the finished products. The samples have a pleasant taste, appearance and colour on the cut; tender and juicy consistency.

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