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DETERMINING THE RELATIONSHIP BETWEEN PARAMETERS OF TEXTURE AND CHEMICAL COMPOSITION OF HIGHLY-EFFICIENT SMOKED SIRLOINS

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ABSTRACT

Food texture can be assessed by organoleptic and instrumental methods or using both methods simultaneously. The sensory assessment should be supplemented by objective methods of the instrumental analysis, which are based on the measurement of physical features. In this connection the aim of the research was to analyze the relationship between instrumentally measured parameters of texture of highly-efficient smoked sirloins and the marked features in the products such as: the content of protein, fat, water and shear force. Studies were conducted on 120 highly-efficient smoked sirloins (the Sopocka Sirloin) which were bought in one shop of a popular chain of shops located in Rzeszów. Texture parameters of smoked sirloins were marked instrumentally using the texture profile analysis (TPA) conducted by texturometer Texture Analyser – CT3 – 25 Brookfield company with the attachment made by a cone of about 30 mm in diameter, about 36 mm long and of the angle of 60^{o}. Next marking of chemical composition was conducted using the analyzer of chemical composition NIR - FoodCheck (Bruins company). There was also conducted the measurement of shear force value was carried out using Warner – Bratzler shear device. Relationships between analysed parameters were set by calculating of cofactors of the Pearson's line correlation.

Keywords: Highly-efficient smoked sirloins, texture, chemical composition, correlation coefficients

INTRODUCTION

One of quality factors of a meat product is its texture which is defined as a whole of mechanical, geometrical and superficial features perceived by mechanical, tactual, and sometimes also by visual and auditory receptors. It is mainly shaped during production of a given food. Superficial features of texture of meat products usually concern sensations caused by the presence of water and/or fat in the product and by the way of their release from test tube. Here the following terms can be singled out: oily, greasy and fatty consistencies (**Juszczak, 2005**; **PN-ISO 11036, 1999**).
Food texture can be assessed by organoleptic and instrumental methods (**Szczepańska *and* Dolik, 2012**) or using both methods simultaneously (**Marzec, 2008a,b**). Sensory assessment is extremely essential, but it is often burdened with a mistake resulting from subjective perception of many features by those who make assessment. Moreover, the assessment demands training of assessing team and ensuring suitable conditions of the assessment. That is why the sensory assessment should be supplemented by objective methods of the instrumental analysis, which are based on the measurement of physical features. Thus instrumental methods are increasingly often used in laboratories and industry. The advantages of their usage are: low costs, higher speed, repeatability of obtained results and the independence of values of these results from the psycho-physical state of people who make the assessment or from the place where the research is carried out (**Szczepańska *and* Dolik, 2012**). The majority of instrumental methods are based on mechanical tests which include measurements of parameters of foods resistance to forces which act on them. The most common method used to assess the texture is the TPA test (profile analysis of texture). It is one of the methods that stimulate conditions the sample in the oral cavity is exposed to (**Bourne, 2002**). Other methods often used to assess that feature are the Kramer's and Warner-Bratzler's tests (the measure of cutting strength values) (**Marzec, 2008a; Guzek *et al.*, 2013**).
The analysis of texture of meat and meat products is most often carried out on the basis of the measure of deformations existing during sample pressing, which also allows determining of such parameters as: hardness, springiness, chewiness or gumminess (**Ostoja *and* Cierach**, **2001**; **Caine *et al.*, 2003; Chang *et al.*, 2010**). Hardness is the essential force in order to reach a required deformation, resilience is the speed of returning from the deformed state to the initial state. Chewiness is the energy necessary to grind down products of stable consistency, and gumminess is energy necessary to plasticize a piece of meat to the state which enables its swallowing. (**Chang *et al.*, 2010**). **Caine *et al.* (2003)** and **Ruiz de Huidobro *et al.* (2005)** observed that parameters of TPA test indicate an essential correlation with organoleptic assessment and with results of measurements of cutting strength values, determined by the Warner – Bratzler test. Also the texture of meat products depends on, among other things, chemical composition (and/or on the recipe) which is characterized by a given product (**Makała *and* Olkiewicz, 1999**; **Dolata, 2001**; **Żochowska-Kujawska *et al.*, 2010).**
In this connection the aim of the research was to analyze the relationship between instrumentally measured parameters of texture of highly-efficient smoked sirloins and the marked features in the products such as: the content of protein, fat, water and shear force.

MATERIAL AND METHODS

Studies were conducted on 120 highly-efficient smoked sirloins (the Sopocka Sirloin) which were bought in one shop of a popular chain of shops located in Rzeszów. Meat products were kept in cooling conditions at the temperature of about 4^{o}C till the time of taking measurements. In the Laboratory of the Department of Processing and Agricultural Commodities of Rzeszów University, from each piece, there were cut out 3 samples in the shape of a cube of sides of 30 mm in order to determine the parameters of texture of studied material.
Texture parameters of smoked sirloins were marked instrumentally using the texture profile analysis (TPA) conducted by texturometer Texture Analyser – CT3 – 25 Brookfield company with the attachment made by a cone of about 30 mm in diameter, about 36 mm long and of the angle of 60^{o}. There was conducted a test of double pressing of samples to 50% of their height. The speed of roller during the test was 2 mm.s^{-1}, whereas the break between pressings was 2 s. The following parameters of texture were measured using the software Texture Pro CT: hardness, adhesiveness, cohesiveness, springiness, gumminess, chewiness and resilience. During serial measurements all texture parameters were counted automatically.

The next stage of research was the determination of chemical composition of smoked sirloins. For this purpose particular samples of material destined for studies were ground three times in the meat grinder using the net whose holes' diameter was 4,0 mm. Next marking of chemical composition was conducted using the analyzer of chemical composition NIR - FoodCheck (Bruins company). It is a computer-controlled spectrophotometer operating in the range of waves of 730 – 1100 nm.
There was also conducted the measurement of shear force value was carried out using Warner – Bratzler shear device. The measurement was conducted on smoked sirloin samples cut out by cork borer of 1 cm in diameter (in the shape of roller). The samples prepared in this way were placed on knives of a fragility gauge and next the value of pressing strength (kg/cm^2) necessary to cut the sample was registered. The mean value of three consecutive measurements was accepted as the final result of of measurement for each smoked bacon samples.
All obtained results were classified and analysed by statistical – mathematic calculation. Straight correlations coefficients between chosen features were placed in tables. Statistical calculation was done based on the STATISTICA PL software, version 10. Relationships between analysed parameters were set by calculating of cofactors of the Pearson's line correlation. The strength of the compound was descriptively determined, depending on absolute values of correlation cofactors as follows (**Górecki, 2011**):

$0 < r < 0.3$ – weak degree of correlation,
$0.3 \le r < 0.5$ – medium degree of correlation,
$0.5 \le r < 0.7$ – significant degree of correlation,
$0.7 \le r < 0.9$ – high degree of correlation,
$r \ge 0.9$ – very high degree of correlation,
$r = 1$ – total correlation.

RESULTS AND DISCUSSION

In table 1 were placed correlation cofactors between instrumentally analysed parameters of texture of highly-efficient smoked sirloins. From that data it turns out that there exist statistically significant relationships between studied features. For example, there was observed a very high and positive relation between hardness 2 a was noticed: hardness 1 (r = 0.945), gumminess (r = 0.965) and chewiness (r = 0.952). Also it was stated that statistically significant relations between gumminess, and chewiness (r = 0.994), hardness 2 (r = 0.965), hardness 1 (r = 0.895), springiness (r = 0.584) and between chewiness, and hardness 1 (r = 0.890). Also significant medium degree of correlation was stated between hardness 1 and such features as: chewiness (r = 0.890), springiness (r = 0.521) and between resilience a: hardness 2 (r = 0.582), adhesiveness (r = 0.432) and chewiness (r = 0.638). The mentioned correlation coeficients are positive and statistically significant (p≤0.05). Also from the data presented in table 1 it results that there exists statistically significant negative relation between adhesiveness and resilience (r = -0.325).
Biller (2013) analyzing the relationship between specific instrumental physico - chemical parameters of model meat products and their shear force observed that there are statistically significant dependencies, but only between some of these features indicated in the raw material and they are mainly related to its type. Also this author stated that studies of relations between these features characterizing such a raw product and the product subjected to roasting allow making products of known and controlled features of colour and texture.
According to **Duda (1998)** meat and its products belong to such a food group where the texture is a predominant quality characteristic beside the taste. That is why the meat industry tries to find new possibilities of its improvement. Most frequently functional additions of the plant, animal, or even microbiological origin are used. First of all, they allow shaping and stabilizing such texture features as: consistency, flexibility and block tying and they may result in texture improvement of meat of lower technological usefulness of the type PSE (**Nielsen, 1995**; ***Zhu.*, 1995**). **Cierach *and* Gral (2005)** carried out the texture analysis of the Żywiecka Sausage and rolled bacon with addition of transglutaminase. These authors showed that addition of that substance to meat products resulted in essential changes of texture parameters values, causing the increase of strength of tearing of slice and hardness.
Dolatowski *et al.* (2003) studied rheological and sensory properties of model meat – fat product, which was produced from beef and pork fat with diversified addition of oat seed after the hydrothermal processing. Those authors stated statistically significant influence on the way of preparing oat seed (type of solution) and the size of addition on that texture parameters of those products, such as: hardness, gumminess, or cohesion (correlation coefficient 0.93≥r≥0.68).
Dolik *and* Kubiak (2013) analysing the results from instrumental measurements of texture profile indicated that it is a universal test which allows conducting marking of selected texture parameters of different foodstuffs in a short time and it perfectly complements sensory tests in this respect.

Table 1 Correlation coefficients between instrumentally analysed parameters of smoked sirloins texture

Specification	Hardness 1	Hardness 2	Adhesiveness	Cohesiveness	Springiness	Gumminess	Chewiness	Resilience
Hardness 1	-	0.945*	0.049	-0.043	0.521*	0.895*	0.890*	0.007
Hardness 2	0.945*	-	0.059	0.214	0.582*	0.965*	0.952*	0.086
Adhesiveness	0.049	0.059	-	0.141	0.432*	0.124	0.150	-0.325*
Cohesiveness	-0.043	0.214	0.141	-	0.290*	0.361*	0.353*	0.443*
Springiness	0.521*	0.582*	0.432*	0.290	-	0.584*	0.638*	-0.006
Gumminess	0.895*	0.965*	0.124	0.361*	0.584*	-	0.994*	0.199
Chewiness	0.890*	0.952*	0.150	0.353*	0.638*	0.994*	-	0.215
Resilience	0.007	0.086	-0.325*	0.443*	-0.006	0.199	0.215	-

*- coefficients significant at the level of p≤0.05

In table 2 there were placed coefficient correlations between instrumentally analysed texture parameters, and chemical composition and shear force of smoked sirloins. These data shows that between these characteristics statistically significant correlations are found only at the medium level. For example, an increase in water content in studied products will cause a decrease of their cohesion (r = - 0.446), gumminess (r = - 0.378), chewiness (r = - 0.354) and hardness in lower degree (r = - 0.260). Also an increase of protein content in smoked sirloins will cause a decrease of values of such parameters of their texture as: cohesion (r = - 0.447), gumminess (r = - 0.317) and chewiness (r = - 0.297). However, an increase in the level of fat in these products will result in an increase in their cohesion (r = 0.487), gumminess (r = 0.389), chewiness (r = 0.367) and hardness (r = 0.255), whereas springiness increase in smoked sirloins causes increase in shear force value (r = 0.348).
According to **Dolata (2002)** the fat in meat products together with protein and water influence the quality of meat in an essential way. It shapes rheological values of filling, texture of ready product, its tastiness and juiciness. Essential fat decrease in the recipe of cured meat products causes the product to become empty in terms of taste and its texture to become more stiff, gummy and floury. The replacement of that element by potato fibre does not cause the improvement of their quality either and unfavourably influences rheological values of fillings and texture of ready products.

Table 2 Correlation coefficients between texture parameters, and chemical composition and shear force of smoked sirloins

Specification	Fat	Protein	Water	Shear force
Hardness 1	0.242	-0.181	-0.256*	0.193
Hardness 2	0.255*	-0.188	-0.260*	0.191
Adhesiveness	0.137	-0.160	-0.132	0.203
Cohesiveness	0.487*	-0.447*	-0.446*	0.096
Springiness	0.083	-0.062	-0.091	0.348*
Gumminess	0.389*	-0.317*	-0.378*	0.143
Chewiness	0.367*	-0.297*	-0.354*	0.139
Resilience	0.093	-0.056	-0.041	-0.213

*- coefficients significant at the level of p≤0.05

According to **Wood's *et al.* (2004)** research conducted on cured meat products the juiciness, tastiness and texture of studied products are strongly related with the fat content. **Miller (2004)** states that the increase in meat tenderness is positively correlated with mid muscle fat content because fat is softer than muscle fibres. Moreover, it protects fibres against fast thermal denaturation and it stops water in meat, thus improving tenderness of heated meat. In studies of that author correlations determined between fat content and TPA hardness and cutting strength were: r = -0.63 and r = -0.30 respectively, regardless of whether fat was marked in raw meat, or in heated one. **Caine *et al.* (2003)** stated that springiness and resilience are strongly connected with the content of intramuscular fat. However, in the studies of **Zając *et al.* (2011) the** correlation between the fat content and springiness was not confirmed. On the other hand, the relations between its amount and springiness (r = -0.41), cohesion (r = -0.42) and chewiness (r = -0.61) were stated.

Żochowska-Kujawska *et al.* (2010) observed that increasing water participation in the filling caused a decrease in hardness, gumminess and modules of springiness and viscosity, and the increase in cohesiveness, adaptability and thermal discharge of sausages made from boars' slight meat. On the other hand, increasing fat addition to filling caused a decrease in hardness, cohesiveness, gumminess and modules of springiness and viscosity, and increase in the plasticity of these sausages.

CONCLUSION

Many statistically significant ($p \leq 0.05$) correlation coefficients were stated between instrumentally marked texture parameters of smoked sirloins. Moreover, the majority of those correlations were at a considerable and high level. A statistically negative and significant correlation coefficient was observed only between adhesiveness and resilience. The increase in water and protein content in smoked sirloins was most frequently connected with decrease in values of majority instrumentally marked texture parameters of these products. However, the stated correlations were at the medium level. On the other hand, the increase in fat content in smoked sirloins was connected with an increase in cohesion, gumminess, chewiness and hardness of these products. With an increase in resilience of smoked sirloins there also increases the value of their shear force.

REFERENCES

Biller E. (2013). Wpływ wybranych cech surowca na wskaźnik zbrązowienia i teksturę modelowego wyrobu pieczonego z mięsa mielonego. Żywność. Nauka. Technologia. Jakość, 1 (86), 43 – 58. http://dx.doi.org/10.15193/zntj/2013/86/043-058

Bourne M.C. (2002). Food texture and viscosity: concept and measurement. Second Ed., Food Sci. Technol., Inter. Series, Acad. Press, New York. http://dx.doi.org/10.1016/b978-012119062-0/50006-1

Caine W.R., Aalhus J.L., Best D.R., Dugan M.E.R., & Jeremiach L.E. (2003). Relationship of the texture profile analysis and Warner-Bratzler shear force with sensory characteristics of beef rib steaks. Meat Sci., 64 (4), 333-339. http://dx.doi.org/10.1016/s0309-1740(02)00110-9

Chang H.J., Wang Q., Zhou G.H., Xu X.L., & Li C.B. (2010). Influence of weak organic acids and sodium chloride marination on characteristics of connective tissue collagen and textural properties of beef semitendinosus muscle. Journal of Texture Studies, 41 (3), 279-301. http://dx.doi.org/10.1111/j.1745-4603.2010.00226.x

Cierach M., & Gral R. (2005). Tekstura i barwa wybranych przetworów mięsnych z dodatkiem transglutaminazy. Inżynieria Rolnicza, 9 (69), 19-26.

Dolata W. (2001). Wpływ warunków kutrowania surowców mięsnych i tłuszczowych na jakość farszów i wędlin. Mięso i Wędliny, 3, 26-30.

Dolata W. (2002). Wpływ częściowego zastąpienia tłuszczu błonnikiem ziemniaczanym na kształtowanie jakości farszów i drobno rozdrobnionych produktów mięsnych. Technologia Alimentaria, 1 (2), 5-12.

Dolatowski Z., Stasiak D., & Pisarek S. (2003). Badania właściwości reologicznych oraz oceny sensorycznej modelowego wyrobu mięsno-owsianego o walorach dietetycznych. Technologia Alimentaria 2 (2), 67-75.

Dolik K., & Kubiak M.S. (2013). Instrumentalny test analizy profilu tekstury w badaniu jakości wybranych produktów spożywczych. Nauki Inżynierskie i Technologiczne, 3 (10), 35-44. http://dx.doi.org/10.15611/nit.2013.3.03

Duda Z. (1998). Technologia mięsa w perspektywie początku XXI wieku – wybrane zagadnienia. Gospodarka Mięsna, 12, 46-51.

Górecki T. (2011). Podstawy statystyki z przykładami w R. Wydawnictwo BTC Legionowo.

Guzek D., Głąbska D., Pogorzelska E., Pogorzelski G., & Wierzbicka A. (2013). Instrumental texture measurement of meat in o laboratory research and on a production line. Adv. Sci. Technol. Res. J., 7 (19), 5-11. http://dx.doi.org/10.5604/20804075.1062329

Juszczak L. (2005). Tekstura żywności. Laboratorium Przemysłowe, 2, 40-44.

Makała H., & Olkiewicz M. (1999). Kształtowanie tekstury produktu mięsnego. Przem. Spoż., 54, 47-49.

Marzec A. (2008a). Tekstura żywności. Część I - Wybrane metody instrumentalne. Przem. Spoż., 2 (62), 12-15.

Marzec A. (2008b). Tekstura żywności. Część II - Wybrane metody sensoryczne. Przem. Spoż., 5 (62), 42-45.

Miller R. K. (2004). Palatability, Encyclopaedia of Meat Sciences, Elsevier Ltd.

Nielsen P. M., (1995). Reactions and potential industrial applications of transglutaminase –Food Biotechnology, 9 (3), 119-156. http://dx.doi.org/10.1080/08905439509549889

Ostoja H., & Cierach M. (2001). Kształtowanie tekstury mięsa wołowego. Inżynieria Rolnicza, 10(30), 261-268.

PN-ISO 11036. (1999). Analiza sensoryczna - Metodologia - Profilowanie tekstury. PKN. 1-22.

Ruiz de Huidobro F., Miguel E., Blazquez B., & Onega E. (2005). A comparision between two methods (Warner-Bratzler and texture profile analysis) for testing either raw meat or cooked meat. Meat Science, 69 (3), 527-536. http://dx.doi.org/10.1016/j.meatsci.2004.09.008

Szczepańska K., & Dolik K. (2012). Ocena tekstury żywności wybrane metody mechaniczne. Przem. Spoż., 5 (66), 38-42.

Wood J.D., Richardson R.I., Nute G.R., Fisher A.V., Campo M.M., Kasapidou E., & Sheard, P.R. (2004). Effects of fatty acids on meat quality: a review. Meat Science, 66 (1), 21-32. http://dx.doi.org/10.1016/s0309-1740(03)00022-6

Zając M., Midura A., Palka K., Węsierska E., & Krzysztoforski K. (2011). Skład chemiczny, rozpuszczalność kolagenu śródmięśniowego i tekstura wybranych mięśni wołowych. Żywność. Nauka. Technologia. Jakość, 4 (77), 103 – 116. http://dx.doi.org/10.15193/zntj/2011/77/103-116

Zhu Y., Rinzema A., Tramper J., & Bol J. (1995). Microbial transglutaminase - a review of its production and application in off processing- Appl. Microbiol. Biotechnol, 44, (3-4), 277-282. http://dx.doi.org/10.1007/bf00169916

Żochowska-Kujawska J., Lachowicz K., Sobczak M., Gajowiecki L., Kotowicz M., Zych A., & Oryl B. (2010). Wykorzystanie mięsa z dzików do produkcji modelowych kiełbas drobno rozdrobnionych ze zmiennym dodatkiem wody i tłuszczu. Żywność. Nauka. Technologia. Jakość, 2 (69), 29 – 39. http://dx.doi.org/10.15193/zntj/2010/69/029-039

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DETERMINATION OF NITRATE AS A SOURCE OF RISK FOR HUMAN IN GROUNDWATERS

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ABSTRACT

We monitored the content of nitrates (NO_3^-) in samples taken from individual groundwater resources in the cadastral territory of the city of Nitra and Zobor (Svorad's spring, spring Šindolka and spring Buganka), also used for human consumption in the period 2012 - 2014. NO_3^- contents were assessed by Photocolorimetric method. We also evaluated the results achieved in relation to the current legislation in this area.
The results of the performed analyzes throughout the period shows that the average concentration of NO_3^- represented in the samples of water from Svorad's spring was 12,6 mg.dm^{-3}, spring Šindolka 38,0 mg.dm^{-3} and spring Buganka 103,1 mg .dm^{-3}
The nitrate concentration did not exceed the limit value in samples from spring Šindolka and spring Svorad. Spring Buganka had this value exceeded by up to 100 % of cases. We do not recommend to use water for human consumption from spring Buganka based on the measured values.

Keywords: Groundwater, quality, nitrates, city of Nitra

INTRODUCTION

The origin, evolution and physical - chemical characteristic of the water cover of Earth - hydrosphere is closely linked with the development of its other parts. Between the earth's mantle and the earth's crust, the hydrosphere, atmosphere, lithosphere and biomass occurs constant exchange of water **(Wen Ling, Saintilan, Rogers, 2009)**, that causes changes its chemical and isotopic composition **(Harris, Hobbs, Higgs, 2006).** This is called hydrological cycle, which represents a stable water cycle on Earth.
An indispensable part of the hydrological cycle and a key element in maintaining wetlands and flows in rivers during dry periods is groundwater. It is the part of subsurface water, which forms a continuous level in the ground and the part that fills the cavity of water-bearing rock irrespective of whether it generates or does not generate continuous level **(Bekes, Moiler, 2009)**. According to the mineralization (total dissolved solids) and gas content are divided groundwaters to normal and mineral.
Processes determining qualitative and quantitative composition of natural waters are the nature of the physical, chemical and biochemical **(Gemitzi, Stefanopoulos, 2011).** Furthermore, the nature of natural waters are also affected by climatic conditions, the overall landscape, density and nature of the settlement and so on. **(Turnbull, Jin, Clancy, 2007).**
We use groundwaters for drinks water in our area. It is representing almost 82,2 %. Drinking water supply is used only from underground water sources in the Nitra region. The town of Nitra is supplied from Ponitrianske group water supply and group water supply of Jelka - Galanta - Nitra. On the legislative front, the requirements for water quality is defined by law no. 355/2007 Coll. on protection, support and development of public health and amending certain laws as amended and Government Regulation No.. 354/2006 Coll., Laying down requirements on water intended for human consumption and quality control of water intended for human consumption as amended by Government Regulation no. 496/2010 Coll..
It is generally believed that the content of NO_3^- in the groundwater in the environmental conditions controlled by activities of microorganisms, i.e., mainly the nitrification and denitrification processes. Precipitation water have nitrate concentrations low in Slovakia, averaging 2,65 mg.dm^{-3} **(Bodiš *et al.*, 2000).** NO_3^- concentration is usually increasing in soil water, mainly as a result of biochemical transformations ammonium (NH_4^+) present in the source (mainly rainfall) water.
Nitrates are in the human body after oral intake rapidly and completely absorbed in the upper small intestine and rapidly distributed in the body. About 25 % is excreted into the saliva, which is partially reduced to nitrite by oral microflora. Bacterial nitrate reduction (NO_3^-) to nitrite (NO_2^-) may be configured in other parts of the digestive tract other than the stomach, where it occurs only at a reduced pH **(Abern, Wise, Wright, 2004)** by the equation:

$$NO_3^- \rightarrow NO_2^- \rightarrow H_2N_2O_2 \rightarrow NH_2OH \rightarrow NH_3 \rightarrow NH_2^-$$

MATERIAL AND METHODS

Due to the fact that there are currently no available literature and data sources with a specific analytical outputs to the population in terms of quality ground water resources in the city of Nitra, we mentioned the issues addressed in our work. Aim of this study therefore was to obtain knowledge about the long-term quality of selected groundwater resources used for human consumption and for drinking purposes in the cadastral area of the city Nitra and Zobor, in terms of nitrate levels in the period 2012 - 2014 with the evaluation of their potential risk to the population. As a source of groundwater were evaluated:

1. Svorad's spring (φ = 48020'47 ", λ = 18005'27 "), located in the woods, on a hiking trail Zobor and Svorad's cave under the hill of Zobor (586.9 m asl). It is situated at the highest altitude (305 m asl) from all sources in the cadastral area Zobor. It is a tourist places frequently visited throughout the year.

2. Spring Šindolka (φ = 48019'50 ", λ = 18005'00 '), is situated on Orava street in Nitra. From the assessment of groundwater resources is at the lowest altitude of 158 m above sea level This spring is a significant of power flow throughout the year. People often use this water to irrigate their crops and also for direct consumption. In the surrounding area of the spring there are several potential polluters (Secondary school for agriculture, road, proximity to agricultural use of land and gardens).

3. Spring Buganka (φ = 48019'50 ", λ = 18006'04 '), located on Panská dolina street close to the restaurant. It is located on private land at an altitude of 214 m above sea level, but access to it is enabled. Water quality is threatened mainly fertilization and used in the surrounding garden and transportation.

We took water samples intended for chemical analysis into polyethylene sample containers of 500 cm^3, which we first rinsed with water and then filled up to the cap. The total nitrate content were assessed by Photocolorimetric method using UV mini 1240 PL.

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RESULTS AND DISCUSSION

We collected and defined the content of nitrate (NO_3^-) in groundwater samples from their individual sources monthly in the period 2012 - 2014. The results were processed in Table 1 which shows that:

Chart 1 Concentration of NO_3^- ($mg.dm^{-3}$) in the period 2012 - 2014 in water spring

WS	OP	Year/month											
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
SS	2012	15,8	14,0	13,1	14,7	12,3	12,3	12,0	12,7	13,2	12,8	12,7	13,4
	2013	11,3	8,4	6,7	8,1	11,6	12,7	15,8	13,1	11,2	10,9	9,7	11,7
	2014	16,2	10,4	12,1	16,3	14,8	15,6	14,8	16,1	14,2	11,0	10,9	12,3
ŠS	2012	41,4	33,5	40,2	41,3	38,2	39,8	36,7	37,1	39,1	39,6	38,9	43,6
	2013	35,1	37,4	42,3	39,4	41,2	44,3	42,1	39,9	36,4	38,5	41,5	40,4
	2014	39,2	36,8	44,4	35,7	24,3	28,6	31,1	25,6	41,2	35,9	29,5	44,2
BS	2012	146,3	124,4	119,6	110,1	122,4	135,3	139,2	128,2	126,1	111,6	98,9	128,8
	2013	95,8	89,6	85,4	80,1	71,9	69,1	73,2	71,9	72,4	83,4	79,4	76,5
	2014	135,8	111,8	98,5	110,3	103,5	89,6	110,8	98,7	115,2	96,3	88,9	116,1

Legend: WS – water spring, OP – Obset parameter, SS – Svorad spring, ŠS – Šindolka spring, BS – Buganka spring

The measured values of nitrate in Svorad's spring were ranged from 12,0 $mg.dm^{-3}$ (July) to 15,8 $mg.dm^{-3}$ (January) during 2012. According to the literature, the toxic effects (particularly in children) depend mainly on the reduction of the nitrite and the subsequent reaction of nitrite with hemoglobin. Nitrate of occurrence of foodborne methemoglobinemia and oxidation of Fe^{2+} to Fe^{3+} in the conversion of hemoglobin (Hb) to a dark brown methemoglobin (MetHb), which is unable to transport oxygen **(Savino, Maccario, Guidi, 2006).**

We found the minimum content of nitrates 6,7 $mg.dm^{-3}$ (March) up to a maximum of 15,8 $mg.dm^{-3}$ (July) in 2013. We had the lowest value recorded in February (10,4 $mg.dm^{-3}$), while the maximum in April - 16,3 $mg.dm^{-3}$ NO_3^- in 2014.

According to the authors **(Erkekoglu, Baydar, 2009)**, to give the nitrate of alimentary methemoglobinemia apply particular factors, such as water with an inadmissible amount of nitrate, the pH of gastric juice, bacterial flora of the upper GIT reducing nitrates, the absorption of nitrate from the gastrointestinal tract into the blood, influence enzyme system reducing methemoglobin to hemoglobin and possible influence of fetal hemoglobin.

Set the average nitrate content found throughout the period 2012 amounted in samples of Svorad's spring 13,2 $mg.dm^{-3}$, in 2013 - 10,9 $mg.dm^{-3}$, during the year 2014 - 13,7 $mg.dm^{-3}$. A source with the lowest NO_3^- content of all monitored sources. To consider for the future is worth to compare also spreading rate (flow) spring and other physical indicators, respectively. meteorological variables (the amount of atmospheric precipitation) that may be related to the issue.

We found a minimum content of NO_3^- in water samples from spring Šindolka in February (33,5 $mg.dm^{-3}$) to 43,6 $mg.dm^{-3}$ in December 2012. The found content of nitrate ranged from a minimum value of 35,1 $mg.dm^{-3}$ (January) to 44,3 $mg.dm^{-3}$ (June) in 2013. The results obtained can be seen that in comparison with the previous assessment of groundwater resources (Svoradov spring) are about twice as high. Clearly, though, the greatest impact on the chemical composition and content of nitrate in groundwater are diffuse sources of pollution, which determine their varying degree of anthropogenic influence **(Bodiš *et al.*, 2000)**. Manifestation of these effects can be multiple increase of nitrate levels compared to background levels.

The measured concentrations ranged from a minimum of 24,3 $mg.dm^{-3}$ (April) up to the maximum established in March (44,4 $mg.dm^{-3}$ NO_3^-) in 2014,. Such concentrations are a result of increased agricultural activity or arise nitrification activity of the bacteria. Important role in fluctuations in the concentration of NO_3^- have on the other hand also seasonal changes in temperature, which in practice excludes biochemical reactions in the winter.

We know from the literature **(Šindelářová, 1985)** that in the plants accumulate in higher concentrations when the plants can not use nitrogen. The cause can be improper temperature, humidity and especially the light conditions. An example can be greenhouse spring vegetables, containing up to several fold higher level of nitrates as a field or garden vegetables **(Valašíková, 2005).**

Nitrates in low concentrations and in non-reducing environment are not for a healthy adult harmful and therefore we can not talk about their primary toxicity. It is associated with their relatively rapid liquidation of the kidney. It is known that up to 12 hours the kidneys excreted about 80 % of the nitrate in middle population and about 50 % in the elderly **(Erkekoglu, Baydar, 2009)**. For the permissible daily doses or with prolonged intake does not cause any disturbance to health, is the FAO / WHO established a daily amount of nitrate equivalent to 5 mg $NaNO_3$ per 1 kg of body weight **(FAO / WHO, 1985)**.

The average nitrate content found in 2012 and 2013 were in water samples from the source spring Šindolka more or less balanced by a concentration. It represented 39,1 $mg.dm^{-3}$ in 2012. It was 39,9 $mg.dm^{-3}$ in 2013 and a slight decrease to 34,7 $mg.dm^{-3}$ during the period of 2014.

The minimum measured content of nitrates was in October (98,9 $mg.dm^{-3}$) to a maximum of 146,3 $mg.dm^{-3}$ in January and December in samples taken from spring Buganka during the year 2012. These high concentrations of nitrates, which reduce to nitrites, excluding formation of methemoglobinemia, may cause the reaction of the secondary and tertiary amines which are present in virtually everywhere (vegetables, meat, milk and cereal products, eggs, beer, wine, medicines, pesticides, etc.) the formation of nitrosamines **(Muro, Stucki, Roback, 2005)**.

The content ranged from 69,1 $mg.dm^{-3}$ in June to 95,8 $mg.dm^{-3}$ (January, February) in the evaluated year 2013. It was found that nitrosamines are able to induce tumor formation in all organs of the body, particularly the gastrointestinal tract, urinary bladder and lymphatic system except bones. Regular intake of vitamin C can be prevented form carcinogenic nitrosamines, and on the other hand, chronically low levels of L-ascorbic acid may increase the disposition **(EFSA, 2010)**.

We had a maximum nitrate levels - 135,8 $mg.dm^{-3}$ measured in January and the minimum - 88,9 $mg.dm^{-3}$ in October in the last year of assessment (in 2014). It can be stated that in terms of toxicological and health assessment is a specific risk group population of children in connection with such detected high levels of NO_3^- in the samples.

Set average nitrate levels observed throughout the period 2012 amounted in samples of spring Buganka 124,2 $mg.dm^{-3}$, in 2013 - 79,1 $mg.dm^{-3}$, during the year 2014 - 106,3 $mg.dm^{-3}$.

The legal framework laying down the requirements for water quality and its control form the content of the Act No.126 / 2006 Coll. on public health and on amendments to certain laws and Government Regulation no. 354/2006 Coll., Laying down requirements on water intended for human consumption and quality control of water intended for human consumption.

Comparing the measured results of the average nitrate content with current legislation, which provides drinking water of the highest limit of 50 $mg.dm^{-3}$ on the basis of indirect toxic effects of nitrates on the human body establishes that the limit in samples of water from Svorad's spring and spring Šindolka are not exceeded. However, it was exceeded in samples of water from spring Buganka in 2012 by an average of 74,2 $mg.dm^{-3}$, about 29,1 $mg.dm^{-3}$ in 2013 and by 56,3 $mg.dm^{-3}$ of NO_3^- in 2014. If we try to show illustratively also exceeding the limit value for nitrate in at least one year we find that, for example in 2014 (when we recorded the highest average, that exceedance), the maximum deviation was recorded in January and July, and on the other hand, the minimum in April and November as documented in chart 1.

Chart 1 Exceeding the maximum limit on the NO_3^- in water from spring Buganka in 2014

Mentioned could be due mainly by the position of spring in relation to fertilization used in the surrounding garden and seductive depth of the collector source. Therefore, the use of water for human consumption from the spring Buganka in terms of the nitrate content is not recommended for drinking.

CONCLUSION

We were assessed the content of nitrates (NO_3^-) in water samples taken from groundwater resources in the administrative territory of the town Nitra and Zobor, which are still used for human consumption. We obtained from the 108 experimental results that:
The total content of nitrates which got into the ground water impact the environmental pollution, agricultural activity or arise nitrification activity of the bacteria ranged on average throughout the period in samples from Svorad's spring from 10,9 to 13,7 $mg.dm^{-3}$, from the spring Šindolka from 39,1 to 39,9 $mg.dm^{-3}$ and spring Buganka from 79,1 to 124,2 $mg.dm^{-3}$.
Absorption of nitrate (NO_3^-) in the human body occurs in the upper small intestine. They are rapidly distributed in the body and then reduced to nitrite (NO_2^-), which are substantially toxicologically dangerous as they react with the hemoglobin and the formation occurs of nitrate alimentary methemoglobinemia.
Besides formation of methemoglobinemia the reduced nitrites by reacting with secondary and tertiary amines, which are present almost everywhere (vegetables, meat, milk, cereal products, eggs, and a.) creation of risk nitrosamines.
When comparing the measurement results with current legislation in the given field and the highest limit value nitrate content in water intended for human consumption, we found that the limit was exceeded in samples taken from spring Buganka, a total of 36 times throughout the period 2012-2014.
Samples of water from the spring Buganka it was on average up to 74,2 $mg.dm^{-3}$ in 2012. This value was exceeded in samples of spring Buganka 29,1 $mg.dm^{-3}$ in the period 2013 and by 56,3 $mg.dm^{-3}$ of NO_3^- in 2014.
Based on this fact in terms of establishing the overall average of the nitrate content, groundwater from a given spring Buganka that has failed in the high limit value in this parameter for drinking purposes is not recommended.

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REFERENCES

BERESFORD, S. 1995. Is nitrate in the drinking water associated with the risk of cancer in the urban U.K. *International Journal of Epidemiology*. 14(1). 57-63. http://dx.doi.org/10.1093/ije/14.1.57
BODIŠ, D. 2010: Pozaďová koncentrácia vybraných ukazovateľov v povrchovej a podzemnej vody Slovenska. Štátny geologický ústav Dionýza Štúra : Bratislava, 2010. ISBN 978-80 89343-43-0.
BODIŠ, D., LOPAŠOVSKÁ, M., LOPAŠOVSKÝ, K. 2000. Chemické zloženie snehovej pokrývky na Slovensku - výsledky 25-ročného pozorovania. Podzemná voda č.2, X. Slovenská hydrogeologická konferencia, Herľany. 162-173.
BOUCHARD, A. 2009. Nitrate contamination of groundwater: sources and potential health effects. 150 p. ISSN 1992-6197
DARRACQ, M., DAUBERT, G. 2007. Cyanotic toddler. *Pediatric Emergency Care,* 2007; 23(3):195–199. http://dx.doi.org/10.1097/pec.0b013e3180330a2c
EFSA. 2010. Panel on Contaminants in the Food Chain (CONTAM). Statement on possible public health risks for infants and young children from the presence of nitrates in leafy vegetables. *EFSA Journal,* 2010; 12(1935):1–42.
ERKEKOGLU, P., BAYDAR, T. 2009. Evaluation of nitrite contamination in baby foods and infant formulas marketed in Turkey. *International Journal of Food Sciences and Nutrition,* 2009; 60(3):206–209. http://dx.doi.org/10.1080/09637480701690311
FAO/WHO. 1985. Food Additives Data Systems. Evaluations by the Joint FAO/WHO Expert Committee on Food Additives 1956-1984 FAO. Food and Nutrition Paper 30/Rev Rome, 1985. 115 s.
KOŽIŠEK, F. 2007. Je vodovodni voda vhodna pro připravu kojenecke stravy? *Praktický lékař* 2007; 87(4):224–227.
KRATOCHVÍL, J, MARTINKOVÁ, V., MASOPUST, J. 2010. Methemoglobinemie. *Urgentní medicína* 2010; 13 (2): 33 – 34.
KRČ, R., IVANOVÁ, Ľ., KRIŽANOVÁ, H., 2007. Grafické zobrazenie časových zmien vybraných ukazovateľov kvality povrchových vôd v Slovenskej republike. Správa. SHMÚ, Bratislava, 2007. 125 s.
LANZ, K. 2001. Príručka: European Environment Bureau (EEB) o vodohospodárskej politike EÚ podľa Rámcovej smernice o vodách. Bratislava : REC pre krajiny strednej a východnej Európy, 2001. 53 s.
MIKE, E., SHAND, P. 2011. Natural Groundwater Quality. 488 p. ISBN: 978-1-4051-5675-2.
MURONE, A, STUCKI, P., ROBACK, M. 2005. Severe Methemoglobinemia due to food intoxication in infants. *Pediatric Emergency Care* 2005; 21(8):536–538. http://dx.doi.org/10.1097/01.pec.0000175452.15793.7e
MŽP SR. 2012. Charakteristika verejných vodovodov podľa akciových spoločností. MŽP SR : 2012. 124 s.
PADO, R. 2001. Kvalita povrchových vôd na Slovensku 1999-2000. Bratislava : SHMÚ. 2001. 490 s.
PIZINGEROVA, K., FREMUTH, J., ŠAŠEK, L. 2011. Akutni methemoglobinemie – zavažna alimentarni intoxikace zeleninou koupenou na trhu. *Pediatra pre prax,* 2011; 12(5):216–219.
SAVINO, F., MACCARIO, S., GUIDI, C. 2006. Methemoglobinemia Caused by the Ingestion of Courgette Soup Given in Order to Resolve Constipation in Two Formula-Fed Infants. *Annals of Nutrition and Metabolism,* 2006; 50(4):368–371. http://dx.doi.org/10.1159/000094301
ŠINDELÁŘOVÁ, J. 1985. Obsah dusičnanov a dusitanov v zelenine. Bratislava : Príroda. 1985, 64 s.
TÖLGYESSY, J., HARANGOZÓ, M., DAXNEROVÁ, O. 2000. Monitoring životného prostredia. Univerzita Mateja Bela : Banská Bystrica 2000. 143 s.
VALŠÍKOVÁ, M. 2005. Niekoľko zásad správneho skladovania zeleniny. *Záhradkár*, 2005; 41(11):64.

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THE MODES OF VITAMIN E ADMINISTRATION AND ITS ROLE ON MEAT QUALITY: A MINI-REVIEW

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ABSTRACT

Vitamin E is a well-established micro-nutrient for all animal species. Vitamin E-based additives are globally used in animal nutrition to prevent vitamin E deficiency and sustain animal health and production. Vitamin E as a lipid-soluble, chain-breaking antioxidant, protects cellular membranes from lipid peroxidation preserving the structural integrity of tissues. Vitamin E has been proposed as an effective method to reduce the oxidative processes in meat. Meat oxidation is the principal reason of the quality deterioration. The primary source of vitamin E is the natural tocopherols found in green plant materials and seeds. Among the synthetic forms of vitamin E, the acetate ester of *all-rac* α-tocopherol (*all-rac* α-tocopheryl acetate) is the most common form of vitamin E supplementations, due to its cost and stability in animal feeds. The route of administration (in feed or intramuscularly injected) plays a key role in enhancing the positive effect of vitamin E treatment. Several researches have proposed the intramuscular injection of vitamin E (dl- α-tocopheryl acetate) as a strategy to: i) reduce vitamin E activity losses in feedstuffs; ii) ensure a more standardized administration in animals; iii) study its effect on growth performance, meat quality characteristics, oxidative stability and shelf life of meat products in different livestock animals. New perspective for the vitamin E supplementation in animal production regard the use of vitamin E as a feasible way to reduce the formation of carcinogenic substances in cooked meat with a consequent beneficial effect on human health.

Keywords: Vitamin E, oxidative stability, meat quality

VITAMIN E: SOURCES AND MAIN FUNCTIONS

Over the past decades, several studies have investigated the importance of an adequate vitamin E status to sustain both animal health and production. This vitamin was recognized as a necessary dietary supplement for growth, reproduction, immune function, disease prevention and tissue integrity (**McDowell *et al.*, 1996**). Vitamin E is the generic term used for all tocol and tocotrienols derivatives that exhibit the activity of α-tocopherol. The tocopherols are exclusively synthesized in photosynthetic organisms including higher plants. Significant amounts are found in all green tissues but predominantly occur in seeds. Vitamin E is abundant in whole cereal grains, particularly in germ, and in byproducts containing the germ; in fact, wheat germ oil was long used as a vitamin E supplement. The α-tocopherol is especially high in wheat germ oil, safflower oil and sunflower oil. Corn and soybean oils contain predominantly γ-tocopherol, as well as some tocotrienols. Cottonseed oil contains both α- and γ-tocopherols in equal proportions (**Zingg, 2007**).
Vitamin E is principally known as a natural antioxidant, it can prevent peroxidative degradation of fats in animal cells and the consequent formation of free radicals. By scavenging reactive oxygen species, molecules that are produced through normal metabolism, vitamin E protects cell membranes against oxidative damage (**Hatfield *et al.*, 2000**). It is well known that oxidation is one of the main causes of quality deterioration in meat products with the development of off flavour, discoloration, formation of toxic compounds, poor shelf life, nutrient and drip losses (**Morrisey *et al.*, 1994; Kasapidou *et al.*, 2012**). In recent years, many studies (e.g. **Macit *et al.*, 2003 a, b**; **Maiorano *et al.*, 2007, 2015 a, b**) were conducted on the effect of vitamin E, supplemented in diet or intramuscular injected, on growth performance, meat quality characteristics, oxidative stability and shelf life of meat products in different livestock animals.
Considering that vitamin E cannot be synthesized by animals, the route and form of vitamin E administration play a key role as they strongly influence the level of serum, plasma and muscle vitamin E concentrations. Methods of providing supplemental vitamin E are: as part of a concentrate or liquid supplement and as an injectable product.
For many years, the primary source of vitamin E was the natural tocopherols found in green plant materials and seeds. In fact, several studies have shown that meat produced by cows fed at pasture contains higher levels of α-tocopherol than cattle-fed high concentrate diets (**Maiorano *et al.*, 2005; De la Fuente *et al.*, 2009; Luciano *et al.*, 2011**). In addition, compared with concentrate-based diets, pasture-based feeding systems generally produce greater concentrations of polyunsaturated fatty acids (PUFA) in muscle, but they also provide greater amounts of natural antioxidants (**Wood *et al.*, 2003**). Comparisons of the effect of concentrate- or herbage-based diets on meat shelf life have frequently shown that feeding systems based on green forages confer on meat a superior resistance to oxidative deterioration due to the greater concentrations of antioxidant molecules in green herbage (**Wood and Enser, 1997**). **D'Alessandro *et al.* (2012)** found that the α-tocopherol content of *semimembranosus* muscle of lambs suckled by pasture-fed mothers was found to be twice as higher as that of the lambs suckled by stall-fed mothers. Even if the content of vitamin E in the sheep milk is very low (6.54 ± 0.25 µmol/l) with a very high variability (**Jelínek *et al.*,1996**), maternal milk might be considered an influential factor on the muscle concentration of vitamin E in lambs, since the pasture represents a relevant source of vitamin E for ruminants (**Morrissey *et al.*, 1998**). However, it must be taken into account that vitamin E content and in general the nutritive value of range forage is influenced in a major way by: stage of maturity, edaphic influences, plant species, climate, animal class, and range condition (**Oelberg, 1956; Duckett *et al.*, 2013**). As already mentioned, tocopherol is abundant in fresh pastures and forage, whereas stability of all naturally occurring tocopherols is poor and substantial losses of vitamin E activity occur in feedstuffs when processed and stored, as well as in manufacturing and storage of finished feeds (**McDowell *et al.*, 1996**). Vitamin E sources in these ingredients are unstable under conditions that promote oxidation of feedstuffs such as heat, oxygen, moisture, oxidizing fats and trace minerals.

INTRAMUSCULAR INJECTIONS OF VITAMIN E: GROWTH PERFORMANCE AND MEAT QUALITY

Animals' growth

In the recent years, several studies (reviewed in **Maiorano *et al.*, 2015a**) have proposed the intramuscular injection of vitamin E (dl- α-tocopheryl acetate) as a strategy to reduce the losses of vitamin E activity in feedstuffs and to ensure a more standardized administration in animals. Vitamin E administration, in diet or by injections, significantly increases the muscle α-tocopherol contents (**Salvatori *et al.*, 2004; Kasapidou *et al.*, 2012**), as also confirmed by our recent study (**Maiorano *et al.*, 2015b**). This latter study showed as well a significant difference in the α-tocopherol concentrations between *longissimus* and *vastus lateralis* muscles of treated lambs (Figure 1). This variation between muscles is probably due to factors, such as functional and metabolic differences of muscle fibers and different fiber fat contents. However, **Barja *et al.* (1996)** found that the increase in the muscle α-tocopherol concentration did not occur in a dose-dependent manner. Usually, with the increasing of the administration quantity, there is an increasing in muscle concentration up to reach an optimal dose, to above which the effect of vitamin E in muscle starts to decrease. In addition, tissue accumulation of α-tocopherol in animals treated or supplemented with vitamin E generally depends by numerous factors such as genotype of animal, dose, feeding time and type of muscle (**Salvatori *et al.*, 2004; Álvarez *et al.*, 2008; Ripoll *et al.*, 2013**).

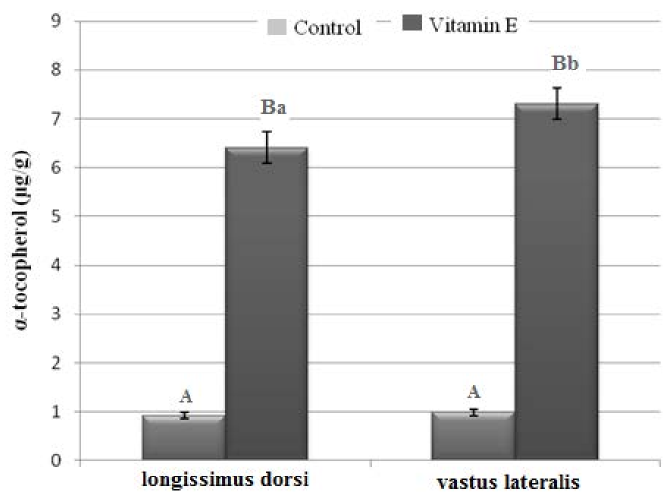

Figure 1 Content of α-tocopherol (μg/g) in *longissimus dorsi* (n=12) and *vastus lateralis* (n=12) muscles between treatments and within treatment (mean±SE). [A, B]$P < 0.01$, between treatments; [a, b]$P < 0.05$, within treatment.

To deepen the knowledge on this topic we will focus on the vitamin E treatment of lambs, which is one of our main research lines. In a recent research, we studied the effects of intramuscular injection of vitamin E on performance, carcass traits, physicochemical and nutritional properties of meat, lipid oxidation, and consumer acceptability in Laticauda lambs, an autochthonous Italian sheep breed (**Maiorano *et al.*, 2015b**). We found that vitamin E treatment did not influence growth but reduced hot dressing percentage. Contradictory data regarding the effect of vitamin E on growth traits exist (**Maiorano *et al.*, 1999; 2007**). Some works have demonstrated a beneficial effect of vitamin E treatment on growth traits in lambs (900 IU DL- α -tocopheryl acetate, **Gentry *et al.*, 1992**; 15 mg vitamin E per lamb per day, **Macit *et al.*, 2003a**). Conversely, other authors (1500 IU α -tocopherol, **Birch *et al.*, 1994**; 1200 IU DL- α –tocopheryl, **Maiorano *et al.*, 2007**) observed a lower carcass weight and a negative effect on carcass wholesale cut weights in lambs injected with vitamin E. In addition, in our recent study (**Maiorano *et al.*, 2015a**) we found that vitamin E did not influence growth, carcass weight, dressing percentage, carcass shrink losses and area of *longissimus* muscle but had a negative effect on pelvic limb percentage. It has been also reported that vitamin E-treated lambs had lower leg and shoulder weights (**Birch *et al.*, 1994**) compared with control lambs. **Hatfield *et al.* (2000)** suggested that this might be due to vitamin E stimulation of the immune system that, in turn, caused a partitioning of energy away from growth and promoted muscle catabolism.

Meat quality

In our recent study (**Maiorano *et al.*, 2015b**) we found that vitamin E treated lambs provided a more intensive colour meat. In fact, vitamin E supplementation delays the discoloration of meat by effectively controlling lipid oxidation and the accumulation of met-myoglobin in meat (**Liu *et al.*, 1995**). This therefore confirms the importance of vitamin E in preserving meat redness that is one of the most important commercial characteristics of meat, because it is the colour that consumers tend to associate with freshness (**Carpenter *et al.*, 2001**). Furthermore, the thiobarbituric acid reactive substances (TBARS) values, found in our research, clearly indicated that injected lambs were best protected from lipid oxidation, in accordance with previous studies on injected lambs (**Salvatori *et al.*, 2004**), in supplemented lambs (**Kasapidou *et al.*, 2012**) and in pork (**Rossi *et al.*, 2013**). This is mainly related to the higher α-tocopherol content in muscles of treated lambs, as confirmed by the negative correlation ($r = -0.46$) between TBARS and muscle α-tocopherol level. Regarding muscle cholesterol content we didn't find any significant effect of vitamin E treatment on it; however, it should be mentioned that vitamin E is associated with reduced production of cholesterol oxidation products which have toxic effects on human health (**Buckley *et al.*, 1995**). On the contrary, a marked effect of vitamin E treatment was found on muscle fatty acid composition. The treatment increased the total PUFA, the PUFA to saturated fatty acids (SFA) ratio and lowered the n-6 to n-3 ratio and the thrombogenic index. **Barja *et al.* (1996)** attributed the increase of PUFA levels in the liver of guinea pigs treated with DL α-tocopherol acetate to a protective effect of vitamin E against PUFA peroxidation. On the other hand, as stated before, the presence of vitamin E within muscle cell membranes reduced lipid oxidation, improving the quality characteristics of meat (e.g. color, flavor, texture and nutritional value) extending its shelf-life, and also preventing the accumulation of toxic compounds, which may be detrimental to the health of consumers (**Falowo *et al.*, 2014**). In light of this, we observed that meat from vitamin E treated lambs received higher hedonic scores for tenderness and juiciness than the respective control. **D'Alessandro *et al.* (2012)** demonstrated that maternal feeding system (stall vs. pasture) affects consumer acceptability of lamb meat. The authors suggested that this effect could be due to higher α-tocopherol content in the meat of pasture feeding lambs.

Intramuscular collagen properties

Tenderness can be explained by different characteristics (e.g. muscle fiber characteristics, intramuscular fat, collagen amount and the stability of its fibers). However, **Sañudo *et al.* (2000)** found no significant differences in tenderness despite differences in fatness. The higher score in tenderness of meat from treated lambs could be due to higher heat soluble connective tissue with cooked meat, favoured by the action of DL-α-tocopheryl acetate that slows collagen maturation and reduce tenderness muscle index (μg of Hydroxylysylpyridinoline crosslinks/mg of intramuscular collagen, **Maiorano *et al.*, 2001, 2007**). In our recent study (**Maiorano *et al.*, 2015a**) conducted on Ile de France suckling male lambs, we found that the DL-α-tocopheryl acetate treatment reduced collagen maturity (HLP/collagen) and increased decorin in the *longissimus* muscle of growing lambs, while it did not affect intramuscular collagen (IMC) content. **Maiorano *et al.* (1999, 2007)** focused on the effect of vitamin E on collagen and demonstrated that intramuscular injections of DL-α-tocopheryl acetate (total dose of 1000 and 1200 IU/animal) given to lambs (slaughtered at 40 days and 70 days old) may increase collagen solubility and reduce the IMC maturity. **Archile-Contreras *et al.* (2011)** reported that vitamin E influences collagen metabolism in animals grown for meat. In particular, they observed that the addition of vitamin E (α-tocopherol acetate) to fibroblast cells, isolated from bovine *semitendinosus* and *longissimus dorsi* muscles, increased significantly total soluble collagen synthesis; while the collagen concentration for neither *semitendinosus* nor *longissimus dorsi* muscle cells responded to vitamin E in a dose-dependent manner. The authors suggested that the results may have implications *in vivo* on animal production, as a high rate of collagen turnover may lead to increased collagen solubility in muscles, which can affect meat tenderness. Collagen, the major component of the intramuscular connective tissue, it is believed to contribute to the initial toughness of meat as well as the sensation of chewiness (**Duizer *et al.*, 1996**). An increase in collagen maturation leads to an increased IMC thermal stability, which has been related to undesirable changes in eating quality of meat (**McCormick, 1999**). It has been reported that vitamin E increases the tenderness of pork (**Dirinck *et al.*, 1996**) and beef

(**Carnagey *et al.*, 2008**), but **Macit *et al.* (2003b)** found that vitamin E supplementation did not alter shear value of meat.

VITAMIN E AND ANIMAL HEALTH

Health is another important aspect of vitamin E supplementation that has to be considered. Vitamin E supplementation program utilizing both parenteral and oral administration is often suggested for livestock animals. Injectable vitamin E (with or without selenium) is available for calves and lambs at birth and 2-4 weeks after birth. Cows and ewes should receive the product 2 weeks prior to parturition. The product is also indicated for early arrival feedlot cattle and lambs as well as those animals which are not consuming adequate vitamin E supplemented diets or which are showing signs of a vitamin E deficiency. For continued protection of all cattle and sheep from a possible vitamin E deficiency, adequate supplemental vitamin E in the feed is essential (**McDowell *et al.*, 1996**). The most common condition associated with vitamin E (and/or selenium) deficiency in lambs, but also in calves and kids, is the white muscle disease. It has also been reported that the deficiency of vitamin E, along with associated nutrients such as selenium and sulfur-containing amino acids, can result in pathological conditions such as encephalomalacia, exudative diathesis, nutritional muscular dystrophy (NMD) in chicks, ducks, and turkeys (**Klasing, 2008**). In light of this, livestock producers will need to employ different strategies to compensate the potential deficiency in the status of vitamin E, considering also the increased nutritional requirements of the fast-growing and high-producing animals. Recent studies have found an emerging meat defect known as "white striping" (WS), which is a new condition affecting poultry breast fillets, principally related to selection for increasing growth rate and breast yield of modern hybrid birds (**Petracci *et al.*, 2013**). The etiological causes of WS are still poorly understood. Considering the observed similarity in the appearance between NMD and WS, **Kuttappan *et al.* (2012)** carried out a study to evaluate the effect of the increments of dietary vitamin E (DL-α-tocopherol acetate: 15, 50, 100, 200, and 400 IU/kg of fed) on the occurrence of various degrees of WS on broiler breast fillets; however, they did not find any significant association in the occurrence of WS in broiler breast fillets. This implies that, in contrast to NMD, an increased level of dietary vitamin E cannot prevent the occurrence of WS. However, it must be taken into account that the requirement of vitamin E may be influenced by various factors affecting the variability of vitamin E in feedstuffs and the physiological status of the birds. Some of these factors are the amount, type, and degree of oxidation of fat present in the diet, presence of other dietary antioxidants such as selenium, iron, copper, sulfur-containing amino acids, and harvesting, drying, or storage conditions of feeds that results in destruction of vitamin E (**Hidiroglou *et al.*, 1992**). In light of this and considering that the intramuscular route could be considered a successful strategy to reduce the losses of vitamin E activity in feedstuffs, we started a new research work, currently in progress, to evaluate the effect of vitamin injection on growth, meat quality and occurrence of WS in fast-growing compared with slow-growing broilers reared indoor or with outdoor access.

NEW PERSPECTIVE FOR THE VITAMIN E SUPPLEMENTATION IN ANIMAL PRODUCTION

In the recent years, several studies (e.g. **Pearson *et al.*, 1992; Balogh *et al.*, 1999**) have demonstrated the ability of Vitamin E, as a potent antioxidant compound, to inhibit the formation of mutagenic/carcinogenic heterocyclic amines (HAs) and polycyclic aromatic hydrocarbons (PAHs). Vitamin E is able to inhibit HA-induced mutagenesis or carcinogenesis as a result of different actions interfering at various steps of the HAs formation and of HA-toxic activity. In fact, antioxidants can act as inhibitors along the different pathways of the reaction, preventing the mutagens formation, through radical quenchers and free radical scavengers activity; as blocking agents, preventing the biotransformation of premutagens into reactive metabolites by inhibiting metabolic activation, by stimulating detoxification enzymes, or by scavenging reactive molecules; as suppressing agents modulating intracellular processes, which are involved in DNA repair mechanisms, tumour promotion and tumour progression (**Vitaglione and Fogliano, 2004**). Our preliminary results, regarding the utilization of vitamin E solution for marinating meat before grill cooking at high temperature, showed the inhibition effect of vitamin E against the formation of carginogenic compounds (**Di Memmo *et al.*, 2012**). However, more studies are needed to further address the inhibition of HAs and PAHs formation in cooked meats by vitamin E (intramuscular injected or from pasture). This clearly involves more detailed studies on the mechanism of HAs and PAHs formation in meat, considering also that the presence of these undesirable substances depends on many factors such as meat type, cooking method, time and temperature, the presence of relative amounts of precursors, enhancers and inhibitors, lipids, antioxidants and the water content. In conclusion, vitamin E as other natural antioxidant substances could be a feasible way to reduce the HAs and PAHs dietary intake with a possible reduction of the risk associated to HAs/PAHs-induced mutagenicity/carcinogenicity with a consequent beneficial effect on human health.

REFERENCES

ÁLVAREZ, I., DE LA FUENTE, J.D., DÍAZ, M. T., LAUZURICA, S., PÉREZ, C., CAÑEQUE, V. 2008. Estimation of α-tocopherol concentration necessary to optimise lamb meat quality stability during frozen storage in high-oxygen modified using broken line regression analysis. *Animal,* 2, 1405−1411. http://dx.doi.org/10.1017/S1751731108002590

ARCHILE-CONTRERAS, A.C., CHA, M.C., MANDELL, I.B., MILLER, S.P., PURSLOW, P.P., 2011. Vitamins E and C may increase collagen turnover by intramuscular fibroblasts. Potential for improved meat quality. *Journal of Agricultural and Food Chemistry,* 59, 608–614. http://dx.doi.org/10.1021/jf103696t

BALOGH, Z., GRAY, J.I., GOMAA, E.A., BOOREN, A.M., 2000. Formation and inhibition of heterocyclic aromatic amines in fried ground beef patties. *Food and Chemical Toxicology,* 38, 395–401. http://dx.doi.org/10.1016/S0278-6915(00)00010-7

BARJA, G., CADENAS, S., ROJAS, C., PÉREZ-CAMPO, R., LÓPEZ-TORRES, M., PRAT, J., PAMPLONA R., 1996**.** Effect of dietary vitamin E levels on fatty acid profiles and non-enzymatic lipid peroxidation in the guinea pig liver. *Lipids*, 31, 963−970. http://dx.doi.org/10.1007/BF02522690

BIRCH, K.S., THOMAS, J.D., ROSS, T.T., 1994. Growth and carcass characteristics of newly received feeder lambs treated with probiotics and vitamin E. *Sheep Goat Research Journal*, 10, 201–206.

BUCKLEY, D.J., MORRISSEY, P.A., GRAY J.I., 1995. Influence of dietary vitamin E on the oxidative stability and quality of pig meat. *Journal of Animal Science,* 73, 3122–3130. http://dx.doi.org/1995.73103122x

CARNAGEY, K.M., HUFF-LONERGAN, E.J., TRENKLE, A., WERTZ-LUTZ, A.E., HORST, R.L. BEITZ, D.C., 2008. Use of 25-hydroxyvitamin D3 and vitamin E to improve tenderness of beef from the longissimus dorsi of heifers. *Journal of Animal Science,* 86, 1649–1657. http://dx.doi.org/10.2527/jas.2007-0502

CARPENTER, C.E., CORNFORTH, D.P., WHITTIER, D., 2001. Consumer preferences for beef color and packaging did not affect eating satisfaction. *Meat Science,* 57, 359–363. http://dx.doi.org/10.1016/S0309-1740(00)00111-X

D'ALESSANDRO, A.G., MAIORANO, G., KOWALISZYN, B., LOIUDICE, P., MARTEMUCCI, G., 2012. How the nutritional value and consumer acceptability of suckling lamb meat is affected by maternal feeding system. *Small Ruminant Research,* 106, 83–91. http://dx.doi.org/10.1016/j.smallrumres.2012.02.001

DE LA FUENTE, J., DIAZ, M.T., ALVAREZ, I., OLIVER, M.A., FONTI FURNOLS, M., SAÑUDO, C., CAMPO, M.M., MONTASSI, F., NUTI, G.R., CANEQUE, V., 2009. Fatty acid and vitamin E composition of intramuscular fat in cattle reared in different production systems. *Meat Science,* 82, 331–337. http://dx.doi.org/10.1016/j.meatsci.2009.02.002

DI MEMMO, D., GAMBACORTA, M., MAIORANO, G., 2012. Preliminary results on the effects of antioxidants and of cooking method on the formation of HCAs and PAHs in cooked meat . International Ph.D. Workshop on "Welfare, Biotechnology and Quality of Animal Production", Zielonka (Poland), 5-8 September 2012.

DIRINCK, P., DE WINNE, A., CASTEELS, M., FRIGG, M., 1996. Studies on vitamin E and meat quality. 1. Effect of feeding high vitamin E levels on time-related pork quality. *Journal of Agricultural and Food Chemistry,* 44, 65–68. http://dx.doi.org/10.1021/jf940607x

DUCKETT, S.K., NEEL, J.P.S., LEWIS, R.M., FONTENOT, J.P., CLAPHAM, W.M., 2013. Effects of forage species or concentrate finishing on animal performance, carcass and meat quality. *Journal of Animal Science,* 91, 1454–1467. http://dx.doi.org/10.2527/jas.2012-5914

DUIZER, L.M., GULLETT, E.A., FINDLAY, C.J., 1996. The relationship between sensory time-intensity, physiological electromyography and instrumental texture profile analysis measurements of beef tenderness. *Meat Science*, 42, 215–224. http://dx.doi.org/10.1016/0309-1740(95)00022-4

FALOWO, A.B., FAYEMI P.O., MUCHENJE V., 2014. Natural antioxidants against lipid–protein oxidative deterioration in meat and meat products: A review. *Food Research International*, 64, 171–181. http://dx.doi.org/10.1016/j.foodres.2014.06.022

GENTRY, P.C., ROSS, T.T., OETTING, B.C., BIRCH, K.D., 1992. Effects of supplemental dl-a-tocopherol on preweaning lamb performance, serum and colostrum tocopherol levels and immunoglobulin G titers. *Sheep Goat Research Journal,* 8, 95–100.

HATFIELD, P.G., DANIELS, J.T., KOTT, R.W., BURGESS, D.E., EVANS, T.J., 2000. Role of supplemental vitamin E in lamb survival and production: A review. *Journal of Animal Science*, 77, 1–9. http://dx.doi.org/10.2134/jas2000.77E-Suppl1a

HIDIROGLOU, N., CAVE, N., ATWALL, A.S., FARNWORTH, E.R., MCDOWELL, L.R., 1992. Comparative vitamin E requirements and metabolism in livestock. *Annals of Veterinary Research*, 23, 337–359. https://hal.archives-ouvertes.fr/hal-00902095

JELÍNEK, P., GAJDUSĚK, S., ILLEK, J., 1996. Relationship between selected indicators of milk and blood in sheep. *Small Ruminant Research*, 20, 53–57. http://dx.doi.org/10.1016/0921-4488(95)00771-7

KASAPIDOU, E., WOOD, J.D., RICHARDSON, R.I., SINCLAIR, L.A., WILKINSON, R.G., ENSER, M., 2012. Effect of vitamin E supplementation and diet on fatty acid composition and on meat colour and lipid oxidation of lamb leg steaks displayed in modified atmosphere packs. *Meat Science*, 90, 908–916. http://dx.doi.org/10.1016/j.meatsci.2011.11.031

KLASING, K.C., 2008. Nutritional diseases. Pages 1027–1054 in Diseases of Poultry. 12th ed. Saif, Y. M., Fadley, A. M., Glisson, J. R., McDougald, L. R., Nolan, L. K., Swayne, D. E. Ames, IA: Blackwell Publishing Professional.

KUTTAPPAN, V.A., GOODGAME, S.D., BRADLEY, C.D., MAUROMOUSTAKOS, A., HARGIS, B.M., WALDROUP, P.W., OWENS, C.M., 2012. Effect of different levels of dietary vitamin E (dl-α-tocopherol acetate) on the occurrence of various degrees of white striping on broiler breast fillets. *Poultry Science,* 91, 3230–3235. http://dx.doi.org/10.3382/ps.2012-02397

LIU, Q., LANARI, M.C., SCHAEFER, D.M., 1995. A review of dietary vitamin E supplementation for improvement of beef quality. *Journal of Animal Science*, 73, 3131–3140. http://dx.doi.org/1995.73103131x

LUCIANO, G., MOLONEY, A.P., PRIOLO, A., ROHRLE, F.T., VASTA, V., BIONDI, L., LOPEZ-ANDRES, P., GRASSO, S., MONAHAN, F.J., 2011. Vitamin E and polyunsaturated fatty acids in bovine muscle and the oxidative stability of beef from cattle receiving grass or concentrate-based rations. *Journal of Animal Science*, 89, 3759–3768. http://dx.doi.org/10.2527/jas.2010-3795

MACIT, M., AKSAKAL, V., AMSEN, E., AKSU, M.L. 2003a. Effects of vitamin E supplementation on performance and meat quality traits of Morkaraman male lambs. *Meat Science*, 63, 51–55. http://dx.doi.org/10.1016/S0309-1740(02)00052-9

MACIT, M., AKSAKAL, V., AMSEN, E., ESENBUGA, N., AKSU, M.I., 2003b. Effects of vitamin E supplementation on fattening performance non-carcass components and retail cuts percentages and meat quality traits of Awassi lambs. *Meat Science,* 64, 1–6. http://dx.doi.org/10.1016/S0309-1740(02)00115-8

MAIORANO, G., ANGWECH, H., DI MEMMO, D., WILKANOWSKA, A., MUCCI, R., ABIUSO, C., TAVANIELLO, S., 2015b. Effects of intramuscular Vitamin E multiple injection on quality, oxidative stability and consumer acceptability of Laticauda lamb meat. Submitted to *Small Ruminant Research*.

MAIORANO, G., CAVONE, C., MCCORMICK, R.J., CIARLARIELLO, A., GAMBACORTA, M., MANCHISI, A., 2007. The effect of dietary energy and vitamin E administration on performance and intramuscular collagen properties of lambs. Meat Science 76, 182–188. http://dx.doi.org/10.1016/j.meatsci.2006.11.001

MAIORANO, G., FILETTI, F., SALVATORI, G., GAMBACORTA, M., BELLETTI, A., ORIANI, G., 2001. Growth, slaughter and intra-muscular collagen characteristics in Garganica kids. *Small Ruminant Research,* 39, 289–294. http://dx.doi.org/ 10.1016/S0921-4488(00)00207-8

MAIORANO, G., MANCHISI, A., SALVATORI, G., FILETTI, F., ORIANI, G., 1999. Influence of multiple injections of vitamin E on intramuscular collagen and bone characteristics in suckling lambs. *Journal of Animal Science,* 77, 2452–2457. http://dx.doi.org/1999.7792452x

MAIORANO, G., PRISCIANTELLI, A., CAVONE, C., GAMBACORTA, M., MANCHISI, A., 2005. Influence of vitamin E treatment starting time on lamb meat quality. *Italian Journal of Animal Science,* 4, 363–365. http://dx.doi.org/10.4081/ijas.2005.2s.363

MAIORANO, G., WILKANOWSKA, A., TAVANIELLO, S., DI MEMMO, D., DE MARZO, D., GAMBACORTA, M., 2015a. Effect of intramuscular injections of DL-α-tocopheryl acetate on growth performance and extracellular matrix of growing lambs. *Animal*, 9, 2060–2064. http://dx.doi.org/10.1017/S175173111500155X

MCCORMICK, R.J., 1999. Extracellular modifications to muscle collagen implications for meat quality. *Poultry Science*, 78, 785–791. http://dx.doi.org/10.1093/ps/78.5.785

MCDOWELL, L.R., WILLIAMS, S.N., HIDIROGLOU, N., NJERU, C.A., HILL, G.M., OCHOA, L., WILKINSON, N.S., 1996. Vitamin E supplementation for the ruminant. *Animal Feed Science Technology*, 60, 273–296. http://dx.doi.org/10.1016/0377-8401(96)00982-0

MORRISEY, P.A., BUCKLEY, D.J., SHEEHY, P.J.A., MONAHAN, F.J., 1994. Vitamin E and meat quality. Proceedings of the Nutrition Society 53, 289–295. http://dx.doi.org/10.1079/PNS19940034

OELBERG, K., 1956. Factors affecting the nutritive value of range forage. *Journal of Range Management*, 9, 220–225. http://dx.doi.org/

PEARSON, A.M., CHEN, C., GRAY, J.I., AUST, S.D. 1992. Mechanism(s) involved in meat mutagen formation and inhibition. *Free Radical Biology and Medicine,* 13, 161–167. http://dx.doi.org/10.1016/0891-5849(92)90078-U

PETRACCI, M., MUDALAL, S., BONFIGLIO, A., CAVANI, C., 2013. Occurrence of white striping under commercial conditions and its impact on breast meat quality in broiler chickens. *Poultry Science*, 92, 1670–1675. http://dx.doi.org/10.3382/ps.2012-03001

RIPOLL, G., GONZÁLES-CALVO, L., MOLINO, F., CALVO, J.H., JOY, M., 2013. Effects of finishing period length with vitamin E supplementation and alfalfa grazing on carcass colour and the evolution of meat color and the lipid oxidation of light lambs. *Meat Science,* 93, 906–913. http://dx.doi.org/10.1016/j.meatsci.2012.09.017

ROSSI, R., PASTORELLI, G., CANNATA, S., TAVANIELLO, S., MAIORANO, G., CORINO, C., 2013. Effect of long term dietary supplementation with plant extract on carcass characteristics meat quality and oxidative stability in pork. *Meat Science*, 95, 542–548. http://dx.doi.org/10.1016/j.meatsci.2013.05.037

SALVATORI, G., PANTALEO, L., DI CESARE, C., MAIORANO, G., FILETTI, F., ORIANI, G., 2004. Fatty acid composition and cholesterol content of muscle as related to genotype and vitamin E treatment in crossbred lambs. *Meat Science*, 67, 45–55. http://dx.doi.org/10.1016/j.meatsci.2003.09.004

SAÑUDO, C., ENSER, M.E., CAMPO, M.M., NUTE, G.R., MARIA, G.A., SIERRA, I., WOOD, J.D., 2000. Fatty acid composition and sensory characteristics of lamb carcasses from Britain and Spain. *Meat Science*, 54, 339–346. http://dx.doi.org/10.1016/S0309-1740(99)00108-4

VITAGLIONE, P., FOGLIANO, V., 2014. Use of antioxidants to minimize the human health risk associated to mutagenic/carcinogenic heterocyclic amines in food. *Journal of Chromatography B*, 802, 189–199. http://dx.doi.org/10.1016/j.jchromb.2003.09.029

WOOD, J.D., ENSER, M. 1997. Factors influencing fatty acids in meat and the role of antioxidants in improving meat quality. *British Journal of Nutrition*, 78, S49–S60. http://dx.doi.org/10.1079/BJN19970134

WOOD, J.D., RICHARDSON, R.I., NUTE, G.R., FISHER, A.V., CAMPO, M.M., KASAPIDOU, E., SHEARD, P.R., ENSER, M. 2003. Effects of fatty acids on meat quality: A review. *Meat Science*, 66, 21–32. http://dx.doi.org/10.1016/S0309-1740(03)00022-6

ZINGG, J.M. 2007. Vitamin E: An overview of major research directions. *Molecular Aspects of Medicine*, 28, 400–422. http://dx.doi.org/10.1016/j.mam.2007.05.004

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THE EFFECT OF THERMAL PROCESSING ON THE CONTENT OF BIOACTIVE COMPOUNDS IN CRANBERRY(*Vaccinium macrocarpon)* FRUITS

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ABSTRACT

Cranberries fruits like other fresh products lose their basic in particular bioactive components with the course of time and therefore there is a need of their immediate fixation. Drying and freezing are the most commonly used methods of extending the shelf life in the processing of these fruits. The aim of the study was to investigate the effect of thermal processing on the content of biologically active compounds in cranberry (*Vaccinium macrocarpon)* fruits. Samples of fresh fruits were frozen (-20ºC) and dried in various temperature condition (59 and 65ºC). Moreover, for comparison market cranberry was used. In the fresh and processed samples of cranberry, the content of vitamin C (Tillmans), total phenolic compounds (Folin-Ciocalteau) and antioxidant activity (FRAP) as well as titratable acidity and anthocyanins were determined. Fresh fruits were characterized by the highest level of all tested parameters. Freezing causes the least possible loss of valuable ingredients. Significant losses of biologically active components in convective dried fruits were temperature-dependent but they were more abundant in antioxidant components than sweetened dried cranberry available in market.

Keywords: Cranberry fruits, thermal processing, bioactive components, antioxidants

INTRODUCTION

The American cranberry (*Vaccinium macrocarpon* Ait.), with an attractive bright red appearance and distinctive flavor, is recognized as a concentrated source of dietary flavonoids, including anthocyanins, flavonol glycosides, and proanthocyanidins (condensed tannins) as well as various phenolic acids (**Caillet *et al.*, 2011**; **Blumberg *et al.*, 2013**). This plant has been known and cultivated for a long time and in comparison to other, its fruits are highly appreciated by the people. Their special benefits come from many health-promoting properties which include antimicrobial (**Lacombe *et al.*, 2010; Côté *et al.*, 2011**), antifungal (**Mckenna *et al.*, 2002**), anti-inflammatory, antioxidant (**Sun *et al.*, 2002**), antiproliferative (**Seeram *et al.*, 2004**), antiedema, diuretic, antipyretic (**Blumberg *et al.*, 2013**).
Quercetin, possibly the most powerful phytonutrient of the cranberry, has been reported to be a growth inhibitor of primary bladder transitional cell cancers in humans (**Lavigne *et al.*, 2007**; **Pappas *and* Schaich, 2009; Gryszczyńska, 2010**). These benefits appear to be principally due to the ability of cranberries to interfere with the adhesion of some bacteria to select cell types and surfaces, i.e. P-fimbriated *Escherichia coli* from adhering to uroepithelial cells, *Helicobacter pylori*, the causative agent of most gastric and duodenal ulcers, to gastrointestinal mucosa, as well as oral pathogens such as *Streptococcus mutans* to tooth hydroxyapatite (**Lacombe *et al.*, 2010**; **Côté *et al.*, 2011**). Recently, research on the effects of cranberries and their components has also been focused on their use in the prevention and treatment of cardiovascular disease(CVD) (**McKay *and* Blumberg, 2007; Dohadwala *et al.*, 2011**).
Due to the presence of benzoic and citric acids, cranberry belongs to the fruit of high durability, but with additional fusing process (drying, freezing, candying) the period of its shelf life can be more extended (**Blumberg *et al.*, 2013**). Moreover, fresh cranberries fruits has pungent taste, which is not desirable by consumers, therefore different processing methods lead to increasing their eligible (**Pappas *and* Schaich, 2009**). Cranberries can be processed into fresh fruit concentrate, sauce products, and juice drinks. The single-strength juice is very acidic (pH 2.5) and unpalatable. So that, since 1930, cranberry juice cocktail, comprising a mixture of cranberry juice (33%), sweetener, water, and added vitamin C, was introduced. Dried cranberry powder formulated in capsules or tablets is also available (**Dorofejeva *et al.*, 2011**).
The aim of the study was to investigate the effect of drying and freezing process on the chemical composition of cranberry fruits available on the Polish market.

MATERIAL AND METHODS

Material

The material consisted of fresh garden cranberry (*Vaccinium macrocarpon* var. Pilgrim) purchased in the shop. Fruits were frozen and dried to a constant weight (**Table 1**). As a control, sample of fresh cranberry fruits stored in refrigerators conditions were used.

Table 1 Characteristics of the sample preparation

sample	temperature	time	used equipment
fresh	4ºC	7 days	refrigerator
frozen	-20ºC	7 days	freezer
I dried	59ºC	144h	home fruit dryer
II dried	65ºC	102h	laboratory dryer

For comparison sweetened dried cranberries (composed of 70% dried fruits, 29% sugar and 1% sunflower oil; with calorific value 294 kcal per 100 g) available in local market were used.

Methods

Determination were made for fresh and processed samples of cranberry fruits. All assays were performed in three independent repetitions.

The content of vitamin C according to Tillmans

10g of crushed fresh and frozen cranberry fruits as well as dried samples (converted into fresh weight - FW) were extracted with 50 mL of 2% oxalic acid solution. The resulting mixture was left in the dark for 10 minutes and then filtered. 10 mL of the filtrate was titrated with a solution of 2,6-dichlorophenolindophenol (DCPIP) until a light pink color. The content of vitamin C was given in mg per 100 g dry weight (DW).

Cranberry extract preparation

For the other determinations cranberry extracts (10% w/v) were used. Cranberry samples (2 g of fresh and frozen and converted into FW amounts of dried cranberries samples) were dipped in 20 mL of distilled water. The obtained solutions were stirred for 20h at room temeperature using a shaker and then filtered through syringe filter with a diameter of 0.2 µm.

Anthocyanins

The content of anthocyanins was determined spectrophotometricaly at various pH **(AOAC Method 2005; Kaniewska *et al.,* 2013)**. The determination principle consists in measurement the difference in absorbance at pH=1 and pH=4.5 at the wavelength λ = 510 nm and λ = 800 nm. Anthocyanins at pH = 1 exist in the form of red flavylium cation, and at pH 4.5 are converted into the form of a colorless pseudo-alkali. As a blank distilled water was used. The content of anthocyanins was calculated by special formula and expressed as % of DW based on cyanidin-3-glucoside (main anthocyanin component of cranberry).

Total content of phenolic compounds (Folin-Ciocalteau)

To 100 µL of obtained extracts, 500µL of Folin-Ciocalteau reagent (Merck, Germany; diluted in the volume ratio 1:10 with distilled water) and 400 µL of 7.5% Na_2CO_3 were added. The resulting solutions were allowed to stand for 90 minutes at room temperature and then the absorbance at the wavelength λ = 760 nm were measured according to the blank. The content of phenolic compounds were calculated using the prepared calibration curve and expressed in mg gallic acid (GAE)/100 g DW **(Piljac-Žegarac *et al.,* 2009)**.

Antioxidant activity (FRAP)

The FRAP reagent contained 10 mM TPTZ (Sigma, USA) solution in 40 mM HCl, 20 mM $FeCl_3$ and 0.3 M acetate buffer pH 3.6 (POCh, Poland), in volume ratio 1:1:10 **(Çelik *et al.,* 2008)**. Aliquots of 200 µL of cranberry extracts (0.1g FW/1mL) were mixed with 1.8 mL of FRAP reagent and the absorbance of the reaction mixture was measured spectrophotometrically at λ = 593 nm after incubation at 37°C for 10 min against blank. 1 mM Trolox (Sigma-Aldrich, USA) was used for the calibration curves and the results were expressed as mM of Trolox equivalent (TE) per 100 g of fruits (DW).

Acidity

Five grams of the shredded fresh, frozen and dried cranberries samples (converted into fresh weight amounts) were heated to boil with 20 mL of water. The whole material was transferred to volumetric flask and made up to 50 mL with distilled water. After 15 min solution was filtered and 10 mL of the filtrate was titrated with 0.1 M NaOH to pH = 8 (measured by pH-meter). Results were calculated for citric acid and showed as g/100 g of DW.

Statistical analysis

Statistical calculations were performed using StatSoft Statistica, 9.0. Pearson's correlation coefficients to assess interaction between tested parameters were calculated.

RESULTS AND DISCUSSION

The antioxidant activity, and phenolic compounds as well as vitamin C contents in fresh and processed cranberries were determined (**Table 2**). Fresh fruits were the richest source of vitamin C and polyphenols, both created their antioxidant activity. Freezing decreased the level of vitamin C by 25%, whereas the phenolic compounds were not changed and antioxidant activity was surprisingly improved by 41%. During convective drying, intensive losses of studied parameters were observed, especially when higher temperature was applied. The vitamin C level was strongly reduced by 62 and 87% during processing in 59 and 65ºC, respectively. Meanwhile the phenolic compouds losses amounted from 9 to 26%, and antioxidant activity was reduced by 2-21%. In comparison sweetened dried cranberries (commercial) contained significantly lower concentration of studied compounds, 5-fold, 3-fold and 3-fold lower for vitamin C, antioxidant activity and phenolic compounds, respectively.

Table 2 Content of bioactive compounds in tested samples (per 100 g dry weight)

Thermal processing	**Vitamin C** [mg/100g DW]	**FRAP** [mmol TE/100g DW]	**Phenolic compounds** [mg GAE/100g DW]
Fresh	208.60±50.51	101.30±0.27	6434.94±78.86
Frozen	156.03±2.67	143.21±2.50	6420.10±0.00
Dried I	78.95±31.90	98.88±5.37	5847.58±105.15
Dried II	26.32±0.00	79.98±2.81	4743.50±115.66
Dried sweetened	41.36± 5.32	31.22±0.53	2234.20±31.54

The obtained results confirmed negative impact of drying in high temperature on the levels of vitamin C in cranberry fruits and are in agreement with other authors findings. **Del Caro *et al.* (2004)**, who studied the effects of drying parameters on two varieties of plums also showed detrimental impact of high temperature on the level of vitamin C. According to **Świderski *and* Waszkiewicz-Robak (2005)** losses of vitamin C as a result of the high temperature application are around 80%. On the other hand, **Pobereżny *and* Wszelaczyńska (2013)** studied the effects of fixation methods on the quality of fruit and demonstrated that freezing is a good way to keep the high content of vitamin C in fruits.

The total phenolics content in studied fresh cranberry reached 865.5 ± 10.6 mg GAE / 100 g FW (6434.94 ± 78.86 mg of GAE / 100 g DW) and slightly decreased during thermal processing. However, research conducted by **Witkowska *and* Zujko (2009)** showed significantly lower levels of polyphenols in Polish small cranberries (*Oxycoccus palustris* Pers.) fruits (2440 ± 321 mg / 100 g DW) in comparison to our results. **Fabisiak *et al.* (2005)** in the studies on the effect of the drying method and temperature on the level of polyphenols in apples have shown that drying at 60 and 70°C resulted in a 35% decrease in the polyphenolic compounds while drying at low temperatures, especially with the use of freeze-drying method, led to significantly lower losses of the tested compounds.

In studies conducted on Polish small cranberry by **Witkowska *and* Zujko (2009)**, the antioxidant activity of cranberry fruits tested by FRAP method was on the lower level (23.63 ± 3,62 mmol TE / 100 g DW) as compared to our findings. However, the confirmation of adverse effects of high temperature on antioxidant property of fruits were also obtained by **Rutkowska *et al.* (2012)**. During studies on the level of bioactive compounds in wild rose fruits dried by a conventional and a freeze-dried methods they showed that the method of air chilling drying (72ºC) caused a greater decrease in bioactive compounds (lower % of DPPH radical inhibition) than in freeze-drying method **(Rutkowska *et al.,* 2012)**.

Total acidity of fresh and dried fruits were analyzed by titration (**Figure 1**). In order to compare the result, all values were calculated to the dry weight (DW), taking into account the water content of the fruit (approx. 87%). The highest total acidity expressed as citric acid equivalent for fresh cranberry (2.21 g / 100 g of FW = 16.5 g / 100 g DW) was observed. The acidity of the fruit in the gentle drying (59°C, 144h) was reduced by 24.6%. Dried sweetened cranberries contained the least amount of organic acids. The acidity of the different varieties of ripe American cranberry fruits identified in a study conducted by **Teleszko (2011)** was at the same level as in the presented study (2.18-2.66 g of citric acid / 100 g FW).

Freezing is one of the best ways of fusing fruit which is confirmed by the results of anthocyanin content **(Figure 2)**. Fruit subjected to freezing have up to 4% more these compounds than fresh cranberries. This may indicate that even short-term storage of fruits in the refrigerator caused small losses of such dye compounds, as compared to fruit preserved by freezing. The content of anthocyanins in dried fruits cranberries is about 54.3 to 90.3% lower than in fresh, the higher the drying temperature the more drastic decrease in the level of anthocyanins was observed. Anthocyanins are labile compounds that are not very resistant to the action of high temperatures. According to literature data, under the influence of heat glycosidic bonds in dye molecules undergo hydrolysis leading to unstable aglycones, which easily oxidize forming brown, high molecular weight compounds (**Krucnar *et al.,* 2014**), who reported anthocyanin losses of 61% caused by 60 s heating at 115°C of highbush blueberries. The obtained results on the level of antocyanins in fresh cranberry fruits (0.841g/100g FW) indicated that the domestic fruits are more abundant source of these colored compounds as compared to **Pappas *and* Schaich (2009)** findings, who indicated the contents of anthocyanin in American cranberries at the level 0.013-0.171 mg / 100 g of FW.

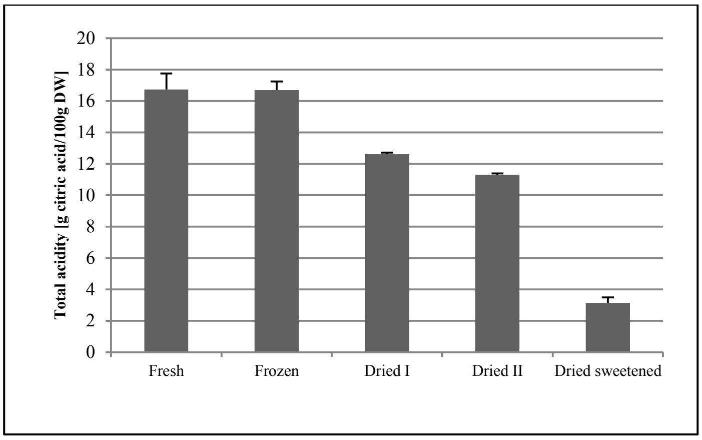

Figure 1 Total acidity of cranberry fruits (g of citric acid/100 g DW) measured by titration

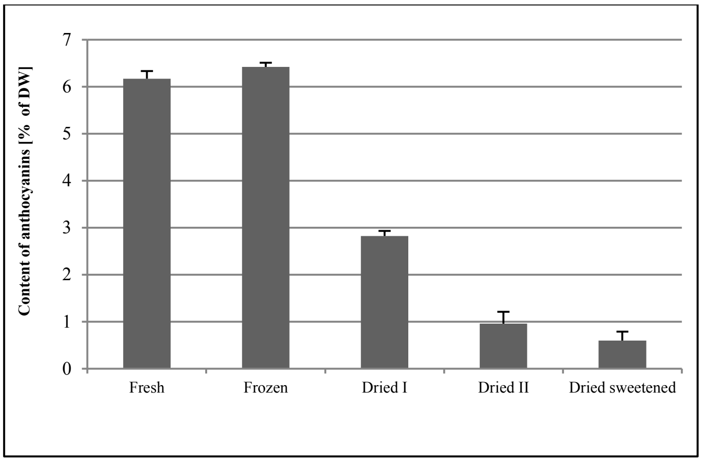

Figure 2 The content of anthocyanin in the cranberry fruits (expressed as cyanidin-3-glucoside)

The comparison of nutritional properties of experimentally processed cranberry fruits and commercial sweetened dried fruits showed that the conventionally dried products have better nutritional properties than commercial one, which was however less acidic. High calorific value of sweetened cranberries and lower level of bioactive compounds, caused that it is sweet snack, dangerous especially for persons with obesity problems.

Among studied parameters significant correlation was observed (**Table 3**). The total antioxidant activity was positively and significantly correlated with the content of phenolic compounds, anthocyanins and vitamin C, indicating that those compounds are responsible for the antioxidant properties of cranberry fruits. The highest correlation (r=0.943) was observed for anthocyanins and vitamin C. It suggests that all tested compounds create antioxidant properties of cranberry fruits.

Table 3 The Pearson's correlation coefficient (r) between analyzed parameters

	FRAP	*Phenolic compounds*	*Acidity*	*Anthocyanins*	*Vitamin C*
FRAP	1				
Phenolic compouds	0.922	1			
Acidity	0.985	0.964	1		
Anthocyanins	0.824	0.833	0.943	1	
Vitamin C	0.724	0.649	0.862	0.955	1

CONCLUSION

Fresh cranberries contains large amounts of bioactive ingredients (vitamin C, polyphenols, anthocyanins), but their contents were reduced during drying process directly proportional to the applied temperature. The best method of cranberry fruits preservation is freezing which does not significantly change their chemical composition. Convective dried fruits possess greater nutritional value than commercial sweetened dried fruits, due to higher content of bioactive compounds as well as lower calorific value.

REFERENCES

AOAC OFFICIAL METHOD, 2005. Total Monomeric Anthocyanin Pigment Content of Fruit Juices, Beverages, Natural Colorants, and Wines. *In: Official Methods of Analysis of AOAC International*, 37, 37-39.

BLUMBERG, J. B., CAMESANO, T. A., CASSIDY, A., KRIS-ETHERTON, P., HOWELL, A., MANACH, C., OSTERTAG, L. M., SIES, H., SKULAS-RAY, A., VITA, J. A. 2013. Cranberries and their bioactive constituents in human health. Review. *American Society for Nutrition,* 4, 618-632, http://dx.doi:10.3945/an.113.004473.

CAILLET, S., CÔTÉ, J., DOYON, G., SYLVAIN, J. –F., LACROIX, M. 2011. Antioxidant and antiradical properties of cranberry juice and extracts. *Food Research International*, 44, 1408-1413, http://dx.doi:10.1016/j.foodres.2011.02.019.

ÇELIK, H., ÖZGEN, M., SERÇE, S., KAYA, C., 2008. Phytochemical accumulation and antioxidant capacity at four maturity stages of cranberry fruit. *Scientia Horticulturae*, 117, 345-348.

CÔTÉ, J., CAILLET, S., DOYON, G., DUSSAULT, D., SYLVAIN, J. –F., LACROIX, M. 2011. Antimicrobial effect of cranberry juice and extracts. *Food Control*, 22, 1413-1418, http://dx.doi:10.1016/j.foodcont.2011.02.024.

DEL CARO, A., PIGA, A., PINNA, I., FENU, P. M., AGABBIO, M., 2004. Effect of Drying Conditions and Storage Period on Polyphenolic Content, Antioxidant Capacity, and Ascorbic Acid of Prunes. *Journal of Agricultural and Food Chemistry*, 52, 4780-4784.

DOHADWALA, M.M., HOLBROOK, M., HAMBURG, N.M., SHENOUDA, S.M., CHUNG, W.B., TITAS, M., KLUGE, M.A., WANG, N., PALMISANO, J., MILBURY, P.E., BLUMBERG, J.B., VITA; J.A., 2011. Effects of cranberry juice consumption on vascular function in patients with coronary artery disease. *The American Journal of Clinical Nutrition*, 93, 934–940, doi: 10.3945/ajcn.110.004242.

DOROFEJEVA, K., RAKCEJEVA, T., GALOBURDA, R., DUKALSKA, L., KVIESIS, J., 2011. Vitamin C content in Latvian cranberries dried in convective and microwave vacuum driers. *Procedia Food Science*, 1, 433-440, http://dx.doi:10.1016/j.profoo.2011.09.067.

FABISIAK, A., SHENG, L., STAWCZYK, J., WITROWA-RAJCHERT, D., 2005. The influence of method and apples drying temperature on the antioxidant activity of extracts produced from those dried apples. *Żywność. Nauka. Technologia. Jakość.* 2, 318-327 (in Polish, abstract in English).

GRYSZCZYŃSKA, A. 2010. Żurawina amerykańska (*Vaccinium macrocarpon*) – lek na problemy urologiczne. *Przegląd Urologiczny* (www.przeglad-urologiczny.pl) (in Polish)

KANIEWSKA, J., GOZDECKA, G., DOMORADZKI, M., SZAMBOWSKA, A., 2013. Processing and characteristic of Kamchatka berries and their products. *Nauki Inżynierskie i Technologie/Engineering Sciences and Technologies*, 4, 1, 58-67 (in Polish, abstract in English).

KUCNER, A., PAPIEWSKA, A., KLEWICKI, R., SÓJKA, M., KLEWICKA, E., 2014. Influence of thermal treatment on the stability of phenolic compounds and the microbiological quality of sucrose solution following osmotic dehydration of highbush blueberry fruits. *Acta Scientarum Polonorum, Technologia Alimentaria*, 13, 1, 79-88

LACOMBE, A., WU, V. C. H., TYLER, S., EDWARDS, K,. 2010. Antimicrobial action of the American Canberry constituents; phenolics, anthocyanins and organic AIDS, against Escherichia coli O157:H7. *International Journal of Food Microbiology*, 139, 102-107, http://dx.doi:10.1016/j.ijfoodmicro.2010.01.035.

LAVIGNE, J.-P., BOURG, G., BOTTO, H., SOTTO, A. 2007. Cranberry (*Vaccinium macrocarpon*) et infections urinaires: étude et revue de la littérature. *Pathologie Biologie*, 55, 460-464, http://dx.doi:10.1016/j.patbio.2007.07.005.

MCKAY, D. L., BLUMBERG, J. B., 2007. Cranberries (*Vaccinium macrocarpon*) and cardiovascular disease risk factors. *Nutrition Reviews,* 65, 490-502, http://dx.doi.org/10.1111/j.1753-4887.2007.tb00273.x

MCKENNA, D.J., JONES, K., HUGHES, K., HUMPHREY, S., 2002. Botanical medicines. The desk reference for major herbal supplements. Second Edition. *The Haworth Herbal Press*, Inc., New York-London-Oxford.

PAPPAS, E., SCHAICH, K. M., 2009. Phytochemicals of Cranberries and Cranberry Products: Characterization, Potential Health Effects, and Processing Stability. *Food Science and Nutrition*, 49, 741-781, http://dx.10.1080/10408390802145377

PILJAC-ŽEGARAC, J., STIPČEVIĆ, T., BELŠČAL, A., 2009. Antioxidant properties and phenolic content of different floral origin honeys. *Journal of ApiProduct and ApiMedical Science*, 2, 43-50, http://dx.10.3896/IBRA.4.01.2.04

POBEREŻNY, J., WSZELACZYŃSKA, E., 2013. Wpływ metod konserwacji na wybrane cechy jakościowe owoców i warzyw znajdujących się w handlu detalicznym. *Inżynieria i Aparatura Chemiczna*, 52, 2, 92-94 (in Polish).

RUTKOWSKA, J., ADAMSKA, A., RUTKOWSKA, J., ADAMSKA, A., PIELAT, M., BIAŁEK, M. 2012. Comparison of composition and properties of *Rosa rugosa* fruits preserved using conventional and freeze-drying methods *Żywność. Nauka. Technologia. Jakość.* 4, 32-43 (in Polish, abstract in English).

SEERAM, N.P., ADAMS, L.S., HARDY, M.L., HEBER, D., 2004. Total cranberry extract versus its phytochemical constituens: antiproliferative and synergistic effects against human tumor cell lines. *Journal of Agriculture and Food Chemistry*, 52, 9, 2512-2517, http://dx.10.1021/jf0352778

SUN, J., CHU, Y.F, WU, X., LIU, R.H., 2002. Antioxidant and antiproliferative activities of common fruits. *Journal of Agriculture and Food Chemistry*, 50, 25, 7449-7454, http://dx.10.1021/jf0207530

ŚWIDERSKI, F., WASZKIEWICZ-ROBAK, B., 2005. Składniki bioaktywne w żywności funkcjonalnej. *Przemysł Spożywczy*, 4, 20-22 (in Polish).

TELESZKO, M., 2011. American cranberry (*Vaccinum macrocarpon* L.) – possibility of using it to produce bio-food. *Żywność. Nauka. Technologia. Jakość*. 79, 132 – 141 (in Polish, abstract in English).

WITKOWSKA, A., ZUJKO, M. E., 2009. Antioxidant activity of wild berries. *Bromatologia i Chemia Toksykologiczna*, 3, 900-903 (in Polish, abstract in English).