VOL I

Estudos em Ciências Agrárias e Ambientais

Eduardo Spers
(Organizador)

EDITORA ARTEMIS
2024

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APRESENTAÇÃO

O campo das ciências agrárias e ambientais está em constante evolução, refletindo a necessidade crescente de entender e gerenciar os recursos naturais e a produção agrícola de maneira sustentável.

O primeiro volume desta nova coletânea "**Estudos em Ciências Agrárias e Ambientais"**, reúne 12 capítulos de destacados pesquisadores, oferece uma visão abrangente das investigações mais recentes em quatro eixos cruciais e complementares: ciências agrárias, ciências dos animais, ciências dos alimentos e ciências ambientais.

No eixo **Estudos em Ciências Agrárias**, os artigos exploram a variabilidade genética e os métodos de cultivo que podem influenciar a produtividade e a qualidade das culturas. O estudo da heterose em sementes híbridas de milho azul (cap. 1) revela como características superiores podem ser obtidas por meio de cruzamentos específicos. Adicionalmente, a análise do potencial genotécnico de híbridos e variedades sintéticas de milho azul (cap. 2) demonstra a importância da adaptação regional para maximizar a produtividade. A pesquisa sobre a manipulação de plantas de limão persa (cap. 3) e a propagação vegetativa do lúpulo (cap. 4) trazem insights sobre práticas de cultivo que podem otimizar a produção.

O eixo **Estudos em Ciências dos Animais** foca na saúde e na eficiência dos sistemas de produção animal. A detecção de imunoglobulinas contra *Anaplasma marginale* (cap. 5) é essencial para a compreensão das doenças bovinas, enquanto a avaliação da eficiência do uso de nutrientes em bovinos (cap. 6) pode melhorar a produtividade e a sustentabilidade das operações de pecuária. O estudo sobre a seroprevalência de *Mycobacterium avium* subespécie paratuberculosis em ovinos (cap. 7) oferece informações valiosas para o controle de doenças em sistemas de produção ovina.

Os artigos do terceiro eixo, **Estudos em Ciências dos Alimentos**, discutem a inovação e a funcionalidade na produção de alimentos. O potencial das sementes de *Moringa oleifera* (cap. 8) é explorado, destacando seus benefícios nutricionais e aplicações alimentares. Além disso, a dinâmica do status total de antioxidantes ao longo do processo de produção de vinho (cap. 9) revela como a qualidade do vinho pode ser monitorada e aprimorada, desde o suco até o produto final.

Finalmente, o eixo temático **Estudos em Ciências Ambientais** aborda questões cruciais relacionadas ao meio ambiente e à conservação. A investigação sobre a doença de manchas marrons e suas interações com hospedeiros (cap. 10) oferece uma visão sobre a gestão de doenças em agroecossistemas. Os avanços na conservação dos recursos genéticos de baunilha no México (cap. 11) são discutidos, evidenciando esforços para preservar espécies ameaçadas e a pesquisa sobre macrofauna bentônica em riachos (cap. 12) demonstra a importância dos organismos do solo para a saúde dos ecossistemas aquáticos.

Este livro não só apresenta pesquisas inovadoras e relevantes, mas também promove uma integração de conhecimentos que é vital para enfrentar os desafios contemporâneos nas ciências agrárias e ambientais. Acreditamos que as descobertas aqui compiladas contribuirão significativamente para o avanço da ciência e para a implementação de práticas mais sustentáveis e eficientes.

Desejo a todos uma proveitosa leitura!

Eduardo Eugênio Spers

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DYNAMICS OF TOTAL ANTIOXIDANT STATUS THROUGHOUT THE WINE PRODUCTION PROCESS: FROM JUICE TO FINISHED NON-ALCOHOLIC WINE PRODUCT

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Daina Garokalna
"Garkalnes mājas vīni" Ltd
Garkalne, Ropaži district, Latvia

Andrejs Skesters
Scientific Laboratory of Biochemistry of Institute of Occupational Safety and Environmental Health of Riga Stradiņš University
Riga, Latvia
https://orcid.org/0000-0003-3726-8724

Anna Lece
Laboratory of Biochemistry of Institute of Occupational Safety and Environmental Health of Riga Stradiņš University
Riga, Latvia
https://orcid.org/0000-0002-0970-2127

Dmitrijs Kustovs
Laboratory of Biochemistry of Institute of Occupational Safety and Environmental Health of Riga Stradiņš University
Riga, Latvia
https://orcid.org/0000-0001-8788-0204

Gundega Gerke
Laboratory of Biochemistry of Institute of Occupational Safety and Environmental Health of Riga Stradiņš University
Riga, Latvia

ABSTRACT: Aim: To quantify the biologically active substances (antioxidants and antiradicals) in berry and fruit juices, and in the resulting wines. Additionally, to determine whether wines lose their antioxidant potential during the de-alcoholization process. **Methods:** We utilized internationally recognized standard analytical methods to measure the levels of biologically active substances. **Results:** Our research allowed us to assess the antioxidant capacity of certain berry and fruit juices. We found that this capacity remains largely unchanged during the winemaking process. Furthermore, wines with low alcohol content or alcohol-free wines produced using our technology retain their antioxidant and antiradical properties. **Conclusion:** Wine is an excellent product containing many antioxidants that can contribute to the regulation of RedOx status in the body. It acts as an effective scavenger and/or inactivator of free radicals and reactive oxygen species, which cause oxidative stress.

KEYWORDS: Antioxidants. Polyphenols. Non-alcoholic wine.

DINÂMICA DO ESTADO ANTIOXIDANTE TOTAL AO LONGO DO PROCESSO DE PRODUÇÃO DE VINHO: DO SUMO AO PRODUTO VINHO NÃO ALCOÓLICO ACABADO

RESUMO: Objetivo: Quantificar as substâncias biologicamente ativas (antioxidantes e antirradicais) em sucos de frutas vermelhas e frutas, e nos vinhos resultantes. Além disso, determinar se os vinhos perdem seu potencial antioxidante durante o processo de desalcolização. **Métodos:** Utilizamos métodos analíticos padrão internacionalmente reconhecidos para medir os níveis de substâncias biologicamente ativas. **Resultados:** Nossa pesquisa permitiu avaliar a capacidade antioxidante de certos sucos de frutas vermelhas e frutas. Constatamos que essa capacidade permanece amplamente inalterada durante o processo de vinificação. Além disso, vinhos com baixo teor alcoólico ou vinhos sem álcool produzidos utilizando nossa tecnologia mantêm suas propriedades antioxidantes e antirradicais. **Conclusão:** O vinho é um excelente produto que contém muitos antioxidantes que podem contribuir para a regulação do estado RedOx no corpo. Ele atua como um eficiente eliminador e/ou inativador de radicais livres e espécies reativas de oxigênio, que causam estresse oxidativo.
PALAVRAS-CHAVE: Antioxidantes. Polifenóis. Vinho sem álcool.

1 INTRODUCTION

The history of wine spans many millennia. It has been known to many cultures in the southern regions, primarily as grape wine. Berry, fruit, and “exotic” wines have a much more recent history. In the modern world, we classify wines by their region of production, grape varieties, growing conditions, climate, production methods, colour, aroma and flavour profiles, sweetness levels, and other characteristics. Additionally, wines can be analyzed based on their bioactive substances, tannins, polyphenols, vitamins, trace elements, and compounds with antioxidant and antiradical effects.

In recent years, wines with reduced or low alcohol content, as well as alcohol-free wines, have gained popularity. Such wines are produced through additional processes that may partially or completely alter their specific properties, including bioactive substances. Both the mutual relations and the quantitative content of these substances can change. This article examines the possible changes in biologically active substances from juice through the winemaking stages to the final wine, including the de-alcoholization process. Preserving the quantity and composition of these biologically active substances is considered a crucial aspect. Modern analytical methods were used in the study to quantify bioactive substances and evaluate their antioxidative (TAS) and antiradical (FRAP) potency.

Many decades ago, Benjamin Franklin famously said, “Wine is proof that God loves us and loves to see us happy!” (*Grigalis U, 2022*). Wine, whether made from grapes, berries, or fruits, is influenced by numerous direct and indirect factors that can significantly affect

the properties of the final product. Wine enthusiasts are divided into two groups: those who believe that good wine comes from high-quality grapes or berries, and those who believe that the winemaker's experience and skills are paramount. In our study, we will not evaluate the skills of winemakers but will focus on the process of making wine from juice to finished wine, including the de-alcoholization process to produce both low-alcohol and non-alcoholic wines.

While the history of well-maintained vineyards dates back to ancient times around 4000 BCE, the history of wines made from other berries, fruits, and exotic sources is much more recent, often only a few centuries old. Different regions use a variety of berries, fruits, and even vegetables for winemaking. Our research includes berries and fruits traditional to the Baltic and Latvian regions. Notably, Latvian gardeners have adapted hundreds of grape varieties to the local climate, thanks to breeders like P. Sukatnieks and A. Fazekas, whose varieties compete in European and global markets. Latvian winemakers, while producing traditional berry, fruit, and exotic wines, are increasingly focusing on grape wines. However, in this article, we will analyze the berry and fruit wines typical of Latvia and the Baltics, focusing on their biological value.

Among the most popular berries and fruits used in Latvian winemaking are: Aronia (*Aronia melanocarpa*), known for its high anthocyanin content (41%) in various forms of cyanidin-glycosides (WICZKOWSKI e colab., 2010), and Raspberries (*Rubus idaeus L.*), containing natural antioxidants like vitamins C and E, flavanols, phenolic acids, ellagitannins, β-sitosterol, and folic acid. The antioxidant properties of raspberries are mainly due to anthocyanins and ellagitannins. Cranberries (*Oxycoccus quadripetalus Gilib.*), Blueberries (*Vaccinium myrtillus L.*), Strawberries (*Fragaria grandiflora Ehrh.*), Gooseberries, Rowanberries (*Grossularia reclinata L.*), Red Currants (*Ribes rubrum L.*), White Currants (*Ribes vulgare Lam.*), Plums (*Prunus sp. L.*), Cherries, Apples (*Malus domestica Brokh.*), Quinces (*Cydonia japonica L.*), and several plants used to make exotic wines, such as Dandelion (*Taraxacum officinale F.H.Wigg.*) and Rhubarb (*Rheum rhaponticum L.*), are also significant. All these plants, whether fruits, berries, or parts thereof, are traditionally used in folk medicine for disease prevention and treatment (*LAGZDINA Z., 2017*).

2 MATERIALS AND METHODS

2.1 MEASUREMENTS OF THE TAS

The Total Antioxidant Status (TAS) in the juice and wine was measured using the Total Antioxidant Status commercial assay kits (Cat. NX2332, Randox Laboratories

Ltd., Crumlin, UK), adapted to the RX Daytona™ automated chemistry analyzer (Randox Laboratories Ltd., Crumlin, UK) following the manufacturer's instructions. Briefly, the assay is based on the formation of the ferryl myoglobin radical from metmyoglobin and hydrogen peroxide, which then oxidizes ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) to produce the radical cation (ABTS•+), a green soluble chromogen, determined spectrophotometrically. Antioxidants scavenge the formed cation-radical in a concentration-dependent manner, leading to a proportional decrease in colour intensity. Assay results are expressed as mmol/L. This measurement considers the cumulative effect of all antioxidants present in the extract under investigation (MILLER e colab., 1993).

2.2 MEASUREMENTS OF THE TPC

The Total Phenolic Content (TPC) in the sapropel extract was determined spectrophotometrically using a UV-Visible spectrophotometer (UV-Vis Varian Cary 50, Seattle, USA) and the widely used Folin–Ciocalteu method (BLAINSKI e colab., 2013; ISO, 2005). This method is based on the oxidation of phenol –OH groups in reaction with the Folin–Ciocalteu reagent, a mixture of phosphomolybdate and phosphotungstate, producing a blue coloration with an absorption at 765 nm. The colour intensity is proportional to the phenol concentration. The reducing capacity of the Folin–Ciocalteu reagent depends on the presence of –OH groups in polyphenols. First, 2.5 mL of 10% Folin–Ciocalteu reagent was added to a 0.5 mL sapropel extract sample, mixed, and incubated at room temperature for 3–8 minutes. Then, 2.0 mL of 7.5% sodium bicarbonate was added, mixed, and incubated for 30 minutes at room temperature. The absorbance was measured at 765 nm against a blank sample. The phenolic compound content in the extract was expressed as gallic acid equivalents (µg GAL/mL). Gallic acid was used to set up a standard curve with concentrations of 100, 50, 25, 12.5, and 6.25 µg GAL/mL. Samples were analyzed in triplicate (BLAINSKI e colab., 2013).

2.3 MEASUREMENTS OF THE FRAP

The Ferric Reducing Antioxidant Power (FRAP) assay was performed according to the method described by Benzie and Strain (BENZIE e STRAIN, 1999). The FRAP reagent was freshly prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ dissolved in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution in a 10:1:1 ratio. Three milliliters of freshly prepared working FRAP reagent were mixed with 0.2 mL of the sample extract. The mixture was incubated at 37°C for 5 minutes. The absorbance was measured at 593 nm against a reagent blank. The FRAP value was calculated and expressed as mM $Fe^{2+} \cdot g^{-1}$

of product, based on a calibration curve plotted using an aqueous solution of 1 mM ferrous sulphate ($FeSO_4 \cdot 7H_2O$) (BENZIE e STRAIN, 1999).

3 RESULTS AND DISCUSSION

In the initial phase, our goal was to monitor the changes in polyphenols, antioxidants (TAS), and antiradicals (FRAP) from juice to the final wine product. We evaluated 18 wines in total, including 11 berry, 4 fruit, and 2 exotic wines. We measured the quantities of polyphenols, total antioxidative capacity, and the ability to bind free radicals (antiradical potencies) in both the juices and the finished wines. The total polyphenolic compounds in the wines were quantified in µg/mL. To determine the polyphenolic compounds, we used a scanning UV/VIS spectrophotometer "Cary 50" (Varian, The Netherlands) and the standardized Folin-Ciocalteu method (ISO 14502-1:2005, 2015), with gallic acid as the standard. The measurement of total polyphenols was expressed as gallic acid equivalent (GAE) µg/mL. Initially, we analyzed the prepared juice before commencing the winemaking process. The qualitative composition of bioactive substances was determined from our previous studies and publicly available data (LAGZDINA , 2017; KUSTOVS e colab., 2020; MOREINO e colab., 2021). Throughout the study, we observed the highest concentrations of polyphenols in chokeberry, blackcurrant, red currant, and quince juice and wine. Chokeberry juice and wine were dominated by anthocyanins and flavanols, including quercetin and kaempferol. In blackcurrant juice and wine, anthocyanins and quercetin primarily determined the antioxidant and antiradical properties. For red currants, quercetin and its derivatives, β-carotenes, lutein, and zeaxanthin contributed to their bioactive potency. Quince juice and wine's bioactive potency comprised anthocyanins, rutin, chlorogenic, and caffeic acids. Antiradical potencies, assessed by the FRAP method, demonstrated the capability of substances in berry and fruit juices and wines to bind and neutralize free radicals and reactive oxygen species. Aronia juice exhibited the highest antiradical potency (11.3 mM Fe^{2+}), followed by blackcurrant (9.9 mM Fe^{2+}), quince (10.5 mM Fe^{2+}), and cherry (8.8 mM Fe^{2+}). The finished wines showed similar antiradical potencies: aronia (11.3 mM Fe^{2+}), blackcurrant (10.1 mM Fe^{2+}), quince (10.0 mM Fe^{2+}), and cherry (9.5 mM Fe^{2+}). These findings convincingly demonstrate that traditional winemaking methods (employed by Latvian winemakers) effectively preserve the bioactive substances responsible for the antioxidant and antiradical properties of wine. This suggests that wine can significantly regulate RedOx processes in the body and reduce oxidative stress by influencing various metabolic processes, including enzymatic and non-enzymatic regulation. A notable

aspect is the wide spectrum of biologically active substances in any wine, enabling each free radical in the body and each reactive form of oxygen and/or nitrogen to find its own "regulator." This contributes to both endogenous antioxidative regulation and exogenous antioxidant defence processes. To substantiate this, we evaluated the total antioxidant potential in juices, finished wines, wines with reduced alcohol content, and non-alcoholic wines. TAS in juice and wine was measured using the Total Antioxidant Status commercial assay kits (Cat. NX2332, Randox Laboratories Ltd., Crumlin, UK) adapted to the RX Daytona™ automated chemistry analyzer (Randox Laboratories Ltd., Crumlin, UK) following the manufacturers' instructions. Aronia juice exhibited the highest antioxidant protection potential (33.2), with aronia wine showing 33.9. Blackcurrant juice and wine showed 19.0 and 18.7, respectively, blueberry 18.0 and 16.8, quince 18.1 and 18.7, and red currant 17.0 and 16.0 (*SKESTERS* e colab*., 2023*). These results justify categorizing wines as preventive agents, as they are known to help in the prevention of cardiovascular diseases and reducing the risk of their development. The results of the study are summarized in Tables 1 and 2 (see below).

Table 1. Total antioxidant status and content of polyphenols and Ferric Reducing/Antioxidant power in juice.

Juice from the following berries	TAS (mmoL/L)	TPC (GAE, µg/mL)	FRAP (mM Fe_2^+)
Aronia *(Aronia melanocarpa)*	33.2	2214	11.3
Black currant *(Ribes nigrum L.)*	19	1606	9.9
Red currant *(Ribes rubrum L.)*	17	1244	7.5
White currant *(Ribes vulgare Lam.)*	4	300	2.7
Rowanberry *(Grossularia reclinata (L.) Mill.)*	3.6	417	4.1
Strawberry *(Fragaria grandiflora Ehrh., Fragaria ananassa Duch.)*	3.7	564	3.8
Cranberry (*Oxycoccus quadripetalus Gilib.*)	5.7	354	4.9
Lingonberry (*Vaccinium vitis-idaea*)	16.8	1146	8
Blueberry (*Vaccinium myrtillus L.)*	18	970	8.1
Raspberry (*Rubus idaeus L., Rubus odoratus*)	12.4	615	7.8
Red grape (*Vitis vinifera*)	12.5	978	6.7
Quince (*Cydonia japonica L., Chaenomeles japonica Thunb*)	18.1	1308	10.5
Cherry (*Prunus avium*)	11.4	952	8.8
Plum (*Prunus sp. L.*)	6.3	440	5.1
Apple (*Malus domestica Brokh.*)	9.4	714	5.3
Dandelion (*Taraxacum officinale F.H.Wigg. s.l.*)	12.8	914	10.7
Rhubarb (*Rheum rhaponticum L.*)	1.2	114	2.2

The total phenolic content of the wine samples was determined using a modified Folin-Ciocalteu method, adapted for a microplate, according to the ISO standard protocol (ISO, 2005). Results were expressed as gallic acid equivalents in µg/mL of wine.

Table 2. Total antioxidant status and content of polyphenols and Ferric Reducing/Antioxidant power in red wine.

Red wine from	TAS mmoL/L	TPC GAE, µg/mL	FRAP
Aronia (*Aronia melanocarpa*)	33.9	2334	11.3
Black currant (*Ribes nigrum L.*)	18.7	1696	10.1
Red currant (*Ribes rubrum L.*)	16	1249	7.9
White currant (*Ribes vulgare Lam.*)	4.2	287	2.6
Rowanberry (*Grossularia reclinata (L.) Mill.*)	3.9	477	4.1
Strawberry (*Fragaria grandiflora Ehrh., Fragaria ananassa Duch.*)	3.2	545	3.9
Cranberry (*Oxycoccus quadripetalus Gilib.*)	6.2	392	4.6
Lingonberry (*Vaccinium vitis-idaea*)	17.4	1286	8.1
Blueberry (*Vaccinium myrtillus L.*)	16.8	1020	8.1
Raspberry (*Rubus idaeus L., Rubus odoratus*)	12.4	615	7.8
Red grape (*Vitis vinifera*)	14.5	1033	7.7
Quince (*Cydonia japonica L., Chaenomeles japonica Thunb*)	18.7	1218	10
Cherry (*Prunus avium*)	10.4	902	9.5
Plum (*Prunus sp. L.*)	6.6	429	4.9
Apple (Malus domestica Brokh.)	7.4	773	5.3
Dandelion (*Taraxacum officinale F.H.Wigg. s.l.*)	12.8	901	10.1
Rhubarb (*Rheum rhaponticum L.*)	1.4	136	2.4

In recent years, there has been a growing trend in society towards increased demand for low-alcohol and non-alcoholic wines, which also impacts winemakers in Latvia. Given the relatively small wine consumption in the Baltic States and Latvia, and the minor share of these specific wine groups in the overall market, it is unfeasible for small wineries to invest in expensive equipment such as reverse osmosis systems costing around $30,000 or spinning cone columns exceeding $1 million (GOODE, 2021) . Our laboratory's objective was to identify and test cost-effective technologies for reducing alcohol content to 8-6% or even zero v/%. One potential method involved "freezing" the wine. The wine was frozen to -30°C, and after removal from the freezer, the bottle was placed upside down in a rack. As the temperature increased, the alcohol, which has a higher freezing point than water, was the first to thaw and slowly flow out. The moment the wine began to melt was determined both visually and photometrically; the first drops

indicated the start of melting. This freeze-thaw procedure was repeated three times during our experiment. After each thawing cycle, the total polyphenol content (TPC) in the wine was measured. The increase in TPC was attributed to the reduced volume of wine due to alcohol separation. The total polyphenol content and alcohol levels in the samples are shown in Table 3. As another method for reducing alcohol content, we used vacuum distillation. Heidolph's vacuum distillation equipment (Heidolph Instruments, GmbH, Germany) allows for precise temperature control (± 0.1°C), standardized rotation speed, and vacuum depth. The distillation process lasted 30 minutes, with a repeated cycle after a 5 minute pause for sampling. The distillation temperature was set at 45°C with a vacuum of 200 millibars. This process was repeated twice, with samples taken for analysis after each step. The results are shown in Table 4.

Table 3. Polyphenols (GAE µg/mL) and alcohol (v/%) content in wine after triple freeze/thaw procedure.

Wine	Original TPC (GAE µg/mL)	Alcohol v/%	1st TPC (GAE µg/mL)	2nd TPC (GAE µg/mL)	3rd TPC (GAE µg/mL)	Final Alcohol v/%
Apple	185	13	193	198	212	6.55
Gooseberry	704	12.5-13.0	716	718	729	4.14
Rhubarb	166	12.5-13.0	175	179	179	3.41
Chokeberry	862	13	870	881	895	8.12

Table 4. Polyphenols (GAE µg/mL) and alcohol (v/v%) content in wine after vacuum distillation.

Wine	Original TPC (GAE µg/mL)	Alcohol v/%	45°C – 200 mbar 30min TPC (GAE µg/mL)	45°C – 200 mbar 30min Alcohol v/%	45°C – 200 mbar 60min TPC (GAE µg/mL)	45°C – 200 mbar 60min Alcohol v/%
Apple	205	12.5-13.0	218	9.7	220	6.8
Rhubarb	130	12.5-13.0	199	2.19	416	0.088
Hawthorn	848	13	859	9.76	872	3.65
Rowanberry	398	13	505	5.6	453	0.66
Apple/pear	227	13	240	2.49	259	0.8

By using the freeze/thaw method, we successfully avoided the loss of biologically active substances, mainly polyphenols. In fact, we observed an increase in polyphenol content per milliliter of wine. This can be explained by the significant reduction in alcohol volume. Despite the considerable drop in alcohol content across all wine samples, most could be classified as low-alcohol wines, except for chokeberry wine, which reached up to 8.12v%. The alcohol content in rhubarb wine decreased from 13.0 to 3.41v%, in gooseberry wine to 4.14v%, and in apple wine to 6.55v%.

The vacuum distillation method also preserved the antioxidant capacity of the wines, similar to the freeze/thaw method. However, the residual alcohol content (ranging from 0.088 to 0.80%) allows these wines to be classified as non-alcoholic. Meanwhile, the alcohol content in hawthorn (3.65v%) and apple (6.8v%) wines places them in the low-alcohol category.

4 CONCLUSION

During the winemaking process, the bioactive substances in the juice are preserved, ensuring the antioxidant capacity of the wine. Our evaluated methods for reducing alcohol in wine or de-alcoholizing wine are gentle and do not diminish the antioxidant potential of low-alcohol or non-alcoholic wines. We believe that wine plays a role in regulating the body's RedOx state, intercepting and neutralizing free radicals, thereby reducing the effects of oxidative stress on the body.

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SOBRE O ORGANIZADOR

EDUARDO EUGENIO SPERS realizou pós-doutorado na Wageningen University (WUR), Holanda, e especialização no IGIA, França. Possui doutorado em Administração pela Universidade de São Paulo (USP). Foi Professor do Programa de Mestrado e Doutorado em Administração e do Mestrado Profissional em Comportamento do Consumidor da ESPM. Líder do tema Teoria, Epistemologia e Métodos de Pesquisa em Marketing na Associação Nacional de Pós-Graduação e Pesquisa em Administração (ANPAD). Participou de diversos projetos de consultoria e pesquisa coordenados pelo PENSA e Markestrat. É Professor Titular no Departamento de Economia, Administração e Sociologia, docente do Mestrado em Administração e Coordenador do Grupo de Extensão MarkEsalq no campus da USP/Esalq. Proferiu palestras em diversos eventos acadêmicos e profissionais, com diversos artigos publicados em periódicos nacionais e internacionais, livros e capítulos de livros sobre agronegócios, com foco no marketing e no comportamento do produtor rural e do consumidor de alimentos.

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