JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

TECHNOLOGICAL, PHYSICOCHEMICAL AND ANTIOXIDANT PROPERTIES OF MALT WORT ENRICHED WITH COCOA SHELLS

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https://doi.org/10.55251/jmbfs.10777

ARTICLE INFO

Received 30. 11. 2023
Revised 7. 1. 2025
Accepted 22. 1. 2025
Published 1. 2. 2025

Regular article

OPEN ACCESS

ABSTRACT

This article discusses the production of malt wort enriched with cocoa bean shells, a by-product of chocolate production, and the subsequent evaluation of its technological, physicochemical, and antioxidant properties. Cocoa bean shells contain dietary fiber and phenols, which are useful for producing low-calorie dietary and fiber-rich products. Addition of 2%, 4%, 6%, 8%, 10%, and 20% cocoa bean shell powder was added to milling malt to prepare the malt wort. A control variant without addition was also prepared to compare the results. The color, density, viscosity, antioxidant activity, total polyphenol content, and nitrogen/protein content of the wort were analyzed. The results showed that the malt wort enriched with cocoa bean shell had higher antioxidant activity and total polyphenol content than the control variant without significant impact on protein content. However, deterioration of quality parameters of the wort as density and viscosity was recorded too. The study provides insights into the potential use of cocoa bean shells as a valuable ingredient in the beverages industry.

Keywords: antioxidant activity, *Theobroma cacao* L., protein, polyphenols, wort, beer

INTRODUCTION

Chocolate is a popular food around the world. However, the production of waste is also associated with cocoa production. There are three by-products of cocoa production: cocoa pod husk, cocoa bean shells (CBS), and cocoa mucilage **(Panak Balentić *et al.*, 2018)**. CBS are produced by chocolate factories before or after roasting the beans **(Beckett, 2011)** and are mainly used as fuel or feedstuff. Shells account for 12-20% of the weight of the cocoa seed, resulting in a global production of 700 thousand tons of waste per year. Shells have interesting properties, with the most important ones being their content of polyphenolics, methylxanthines, lipids, and dietary fiber **(Okiyama *et al.*, 2017)**. The high content of dietary fiber and phenols make them suitable for use in low-calorie dietary and fiber-rich products **(Nsor-Atindana, 2012a,b; Vītola and Ciproviča, 2016)**. Several studies have been published on the use of shells in corn snacks **(Jozinović *et al.*, 2019)**, muffins, and biscuits **(Panak Balentić *et al.*, 2018; Sánchez *et al.*, 2010)**.

Polyphenols are present in many food sources, including fruits, vegetables, cereals, tea, coffee, wine, beer, and extra-virgin olive oil **(Puupponen-Pimiä *et al.*, 2002)**. Many studies have attempted to link the observed health effects of a diet rich in fruits and vegetables with the content of polyphenols in these edible products **(Bohn, 2014; Quesada-Molina *et al.*, 2019)**. Polyphenols have been shown to display a wide range of biological activities, including reductions in markers of inflammation **(Magni *et al.*, 2018)**, inhibitions of the expression of adhesion molecules **(Martínez *et al.*, 2013)**, reductions of oxidative stress **(Fogliano *et al.*, 2011)**, and improvements in anticancer markers **(Mabrok *et al.*, 2012)**. Specific beer-derived polyphenols have also been found to have anti-diabetic **(Costa *et al.*, 2017)**, anti-carcinogenic **(Jiang *et al.*, 2018)**, and anti-inflammatory effects **(Everard *et al.*, 2012)**.

The nutritional benefits of CBS were explored in published studies, revealing that they have similar nutritional values to cocoa butter **(Cinar *et al.*, 2021)**. Further research has been conducted to extract phenolic compounds from CBS, mainly consisting of catechins, epicatechins, and procyanidins. A study conducted by **Okiyama *et al.* (2018)** showed that CBS could also serve as a significant source of flavanols and alkaloids when extracted using the pressurized liquid extraction technique with ethanol. CBS has also been identified as a good source of various minerals, such as potassium, magnesium, calcium, phosphorus, copper, and zinc **(Bonvehí and Jordà, 1998)**. Moreover, research has found that the dominant polyphenols in CBS from different cocoa genotypes are catechin and epicatechin, with theobromine as identified as the major methylxantine component **(Hernández-Hernández *et al.*, 2019)**. CBS has several nutritional benefits that make them an excellent alternative to cocoa butter in food and cosmetic products **(Cinar *et al.*, 2021)**.

Pure beer contains prenylated flavonoids, phenolic acids, and simple phenols that come mainly (70%) from malt **(Ambra *et al.*, 2021; Salanță *et al.*, 2020)**. These compounds contribute to the color, taste, haze, astringency, colloidal, and foam stability of beer. However, some of these compounds are lost during beer processing, so adding other phenols with potential health benefits is appropriate **(Chacón-Figueroa *et al.*, 2022)**.

The aim of this study was to prepare malt wort enriched with 2, 4, 6, 8, 10, and 20% cocoa bean shell and subsequently evaluate its technological, physicochemical, and antioxidant properties. A control group without any additions was also prepared to compare the results, and a pure 100% CBS extract was obtained using a congress mash.

MATERIAL AND METHODS

Materials

Cocoa shells were obtained from the Criollo variety of cocoa beans (Peru, purchased from a market) as a waste product from chocolate production. Before analysis, the cocoa shells were milled into powder using a mixer (Sencor SCG 1050 WH, Japan).

All chemicals used were of analytical grade and were purchased from Sigma-Aldrich (St. Louis, USA) and CentralChem (Slovak Republic).

Methods

Malt wort preparation

The cocoa shell powder was added to milling malt in ratios of 0, 2, 4, 6, 8, 10, and 20%. Certified congress mash (method 4.5.1 Congress Mash EBC) **(Solgajová *et al.*, 2024)** in a Mash Bath-R8,1-CUBE (Czech Republic) and Finest Pale Ale Golden Promise malt (Simpson, Great Britain) were used. The malt was ground, and 50 g ± 0.05 g was weighed into a mashing vessel. Subsequently, 150 ml of distilled water at 45 °C was added, followed by rinsing the stirrer with an

additional 50 ml of distilled water at the same temperature. The mashing vessel was placed in a water bath preheated to 45 °C, and stirring was initiated.
The mash was stirred for 30 minutes at 45 °C. The temperature was then gradually increased by 1 °C per minute until reaching 70 °C within 25 minutes. An additional 100 ml of distilled water at 70 °C was added, and the mixture was stirred for another 10 minutes. A sample was taken for starch hydrolysis testing using iodine. The mash was maintained at 70 °C for 1 hour, then cooled to 20 °C using cold water over 10–15 minutes.
The mash was transferred, rinsed with distilled water, and adjusted to a total weight of 450 g with distilled water. After thorough mixing, the mixture was filtered. The first 100 ml of filtrate was re-filtered, and the filtration process continued until the wort solids were retained, or after a maximum of 2 hours. The filtrate was homogenized.

Determination of color

Color was measured using the spectroscopic method. Wort was filtered to a haze under 1 unit EBC. Absorbance at 430 nm was measured using a spectrophotometer (Cary 60 UV-vis, Agilent Technologies, USA) and multiplied by 25 to obtain a value of color in EBC units (EBC method 8.5).

Determination of density and viscosity

Density and viscosity were measured using an Anton Paar Beer Analyzer Density meter DMA 4500M and Microviscometer Lovis 2000 ME (Anton Paar, Austria).

DPPH method – Radical scavenging activity

Radical scavenging activity of samples was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to the procedures described by **Sanchez-Moreno *et al*. (1998)**. An amount of 0.4 mL of sample was added to 3.6 mL of DPPH solution (0.025 g DPPH in 100 mL ethanol). Absorbance of the reaction mixture was determined using a spectrophotometer (Cary 60 UV-VIS, Agilent Technologies, USA) at 515 nm. Radical scavenging activity of the samples was expressed as Trolox equivalent antioxidant capacity (mg TEAC/L).

Total polyphenol content

Total polyphenol content was measured in accordance with **Singleton and Rossi (1965)** using Folin-Ciocalteu reagent. A 0.1 mL of sample was mixed with 0.1 mL of the Folin-Ciocalteu reagent, 1 mL of 20% (w/v) sodium carbonate, and 8.8 mL of distilled water and left in darkness for 30 min. The absorbance at 700 nm was measured using a spectrophotometer (Cary 60 UV-VIS, Agilent Technologies, USA). Gallic acid (25-300 mg/L; R2=0.998) was used as a standard and the results were expressed in mg/L of gallic acid equivalent.

Nitrogen/protein content

Nitrogen content was measured using the semi-micro Kjeldahl method. The nitrogen was then converted to protein using the conventional factor of 6.25. A sample weighing 0.5 g was mixed with 15 mL of 98 % N-free H_2SO_4 and K_2SO_4+$CuSO_4$ (10:1 w/w). The mixture was then mineralized in a DK6 heating digester (VELP Scientifica, Italy). The distillation was performed with the surplus of NaOH in the distillation unit (UDK 129, VELP Scientifica, Italy) for 5 minutes. The resulting mixture was titrated with Tashir's indicator to 0.05M H2SO4. The results were expressed as the percentage of nitrogen substances per 100 mL **(Šimora *et al*., 2023)**.

Statistical analysis

All experiments were carried out in triplicate, and the mean of replications with standard deviations was reported. The results were statistically processed with XLSTAT (Addinsoft, Paris, France). The experimental data were subjected to analysis of variance using the Dunnett two-sided test, and a summary of all pairwise comparisons was performed using the Tukey test.

RESULTS AND DISCUSSION

Color

A range of *colors* from 4.67 to 14.52 was observed **(Figure 1)**, pure CBS extract had color 46,96±13,75 units EBC. Each addition had a highly significant impact on color change, and the color grew constantly. Comparable results were obtained with the use of roasted malt **(Hoff *et al*., 2012)**. Similarly, we can obtain similar results with commonly used roasted or caramelized malts in the mash.

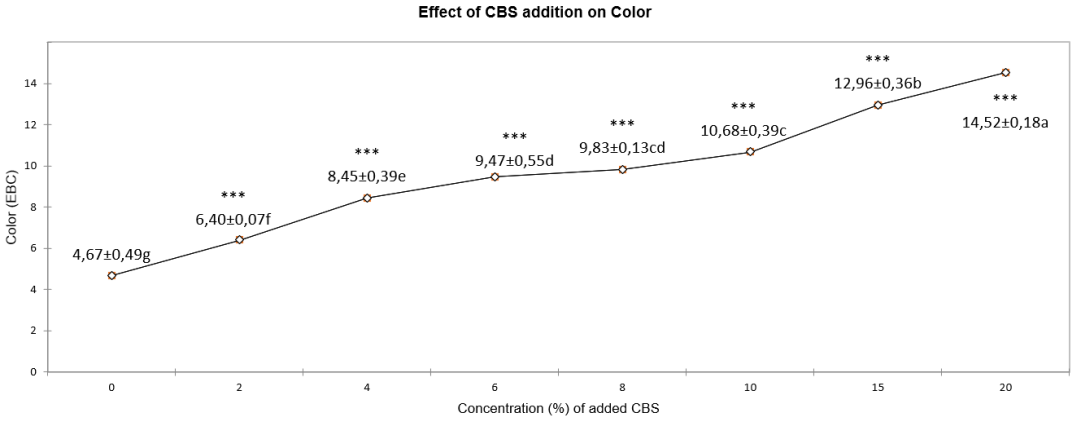

Figure 1 Effect of CBS addition on Color

Density

The density ranged from 1.0337 g/cm³ to 1.03 g/cm³ **(Figure 2).** The density of the pure extract in the congress mash was 1.0104±0.0002 g/cm³. Therefore, adding more CBS as an adjunct into the mash decreased the density of the final wort consistently, which is consistent with other studies **(Bogdan and Kordialik-Bogacka, 2017)**. Adjuncts used in brewing typically lack the hydrolytic enzymes necessary for the degradation of complex carbohydrates and proteins **(Schnitzenbaumer and Arendt, 2013)**. Additionally, adjuncts have compact structures that are not as easily modified as malt, which can hinder enzyme action. When adjuncts are added to the mash vessel, they can contribute to decreased extract yield in the wort due to the lack of available enzymes. The use of adjuncts in place of malt also results in a smaller pool of enzymes involved in the hydrolysis of malt components such as starch, proteins, and cell wall components. As a result, the inclusion of adjuncts in the brewing process can negatively impact overall mash efficiency and lead to lower yields of fermentable sugars in the wort **(Bogdan and Kordialik-Bogacka, 2017; Van Donkelaar *et al*., 2015)**.

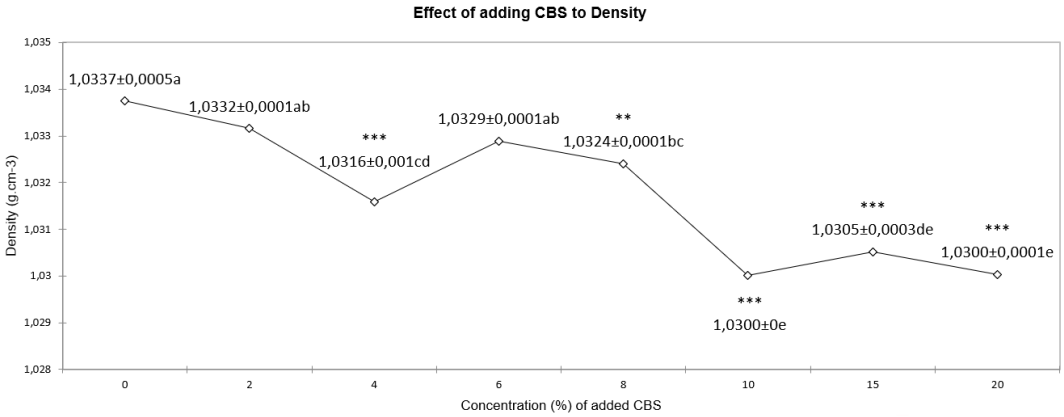

Figure 2 Effect of adding CBS to Density

Viscosity

Viscosity ranged from 1.496 to 1.650 mPa.s **(Figure 3)**. Pure CBS extract was not measured due to its high lipid content, and the measuring device is not constructed to measure lipidic samples. The first significant effect of CBS addition on viscosity was observed at the addition of 8% CBS, when the viscosity value increased to 1.531 mPa.s, then the value increases at the addition of 15 and 20% (1.650 mPa.s). The only exception to the trend is the addition of 10% CBS with a value of 1.517 mPa.s. The content of pectin in cocoa has been extensively studied as it poses technological challenges in the application of cocoa in beverages. Pectin, which is concentrated in various parts of cocoa, increases the viscosity of the beverage and results in losses in the secondary stage. Researchers have studied ways to reduce the impact of pectin in pectin-rich sources during food processing. For instance, in mango pulps, a 5.7% reduction in pectin led to a 50% decrease in viscosity **(Manohar *et al*., 1990)**. In the cocoa field of application, pectolases were used to achieve a 65% reduction in viscosity when using cocoa pulp in beer. The resulting beer had better fermentation parameters and a richer taste profile **(Nunes *et al*., 2020)**.

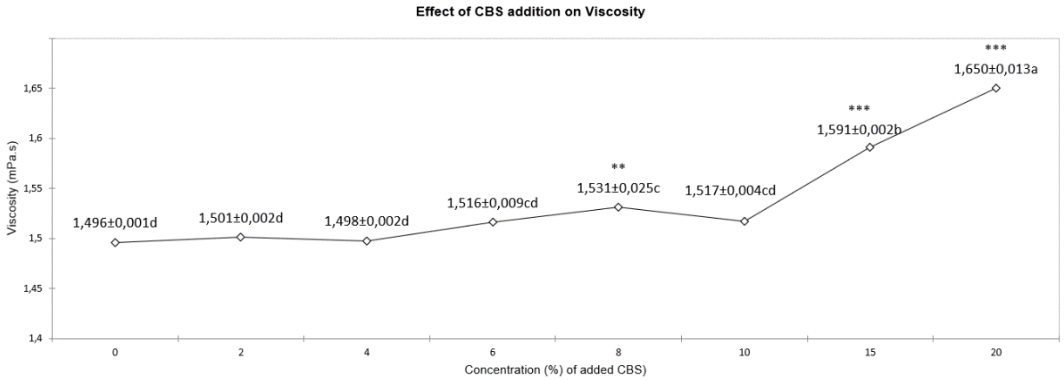

Figure 3 Effect of CBS adding on Viscosity

Antioxidant activity

Antioxidant activity ranged from 73.77 to 81.63 mg TAEC/L **(Figure 4)**, with the highest activity observed in the sample with 8% CBS addition. The results indicate that increasing the concentration of CBS in the wort results in an antagonistic effect, which is consistent with the findings of **Chacón-Figueroa *et al*. (2022)**. In their study, a concentration of coffee bean bagasse extract above 1 mg/mL resulted in decreased antioxidant activity. While the decrease in our experiment was not significant, even a 20% addition did not exhibit as high antioxidant activity as the 8% addition did. Furthermore, the pure extract of CBS had an antioxidant activity of only 75.91±1.06 mg TAEC/L, indicating the possible synergistic effects of bioactive compounds in the wort and CBS or the formation of new antioxidant-active compounds during mash hydrolysis.

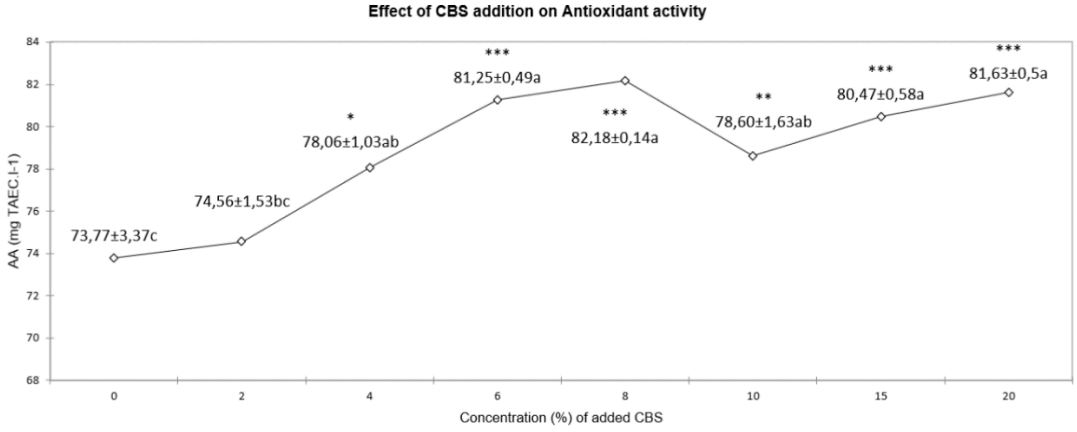

Figure 4 Effect of CBS adding on Antioxidant activity

Total phenolic content (TPC)

Total phenolic content ranged from 297.6 to 423.6 mg GAEL/L **(Figure 5)** in our samples, and the pure extract had a volume of TPC of 772.2±26.7 mg GAEL/L. We observed 2 peaks of TPC concentration in individual additions. The first peak was recorded at the addition of 6% CBS (370 mg GAEL/L), followed by a decrease at the addition of 8 and 10%. The highest value was measured at the addition of 20% CBS (423.6 mg GAEL/L). These results contradict the findings of the study by **Chacón-Figueroa *et al.* (2022)**, where an antagonistic effect was observed in both the field of antioxidant activity and TPC. In our study, the TPC in the pure extract was significantly higher than the content in the wort samples, and the TPC content did not correlate with antioxidant activity. Our data corresponded with results of polyphenol content of pure beer from Database on Polyphenol Content in Food (ranged from 120 to 520 mg.l^{-1}) **(URL1)**.

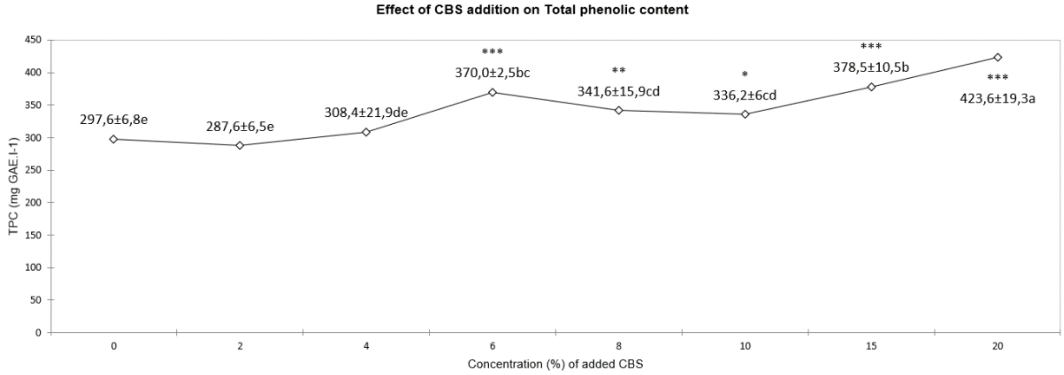

Figure 5 Effect of CBS addition on Total phenolic content

Protein content

Protein content ranged from 59.5 to 66.5 mg Ns.100ml-1 **(Figure 6)**. The highest volume of protein was recorded in the sample without CBS addition. Pure CBS extract had 63.93±3.86 mg Ns.100ml-1. Additions had an impact on protein content, but a significant decrease was recorded only in one sample (4% CBS addition). The content of protein compounds in CBS was studied in the study by **Caprioli *et al.*, (2016)** with different varieties of cocoa, and it was present in amounts ranging from 11.7% to 13.35%. Meanwhile, the content of protein in barley malt is 10-12% **(Jaeger *et al.*, 2021)**, so there are not any big differences in protein content between these two sources, which can predict a decrease in the protein content of the final product, and our results confirm it.

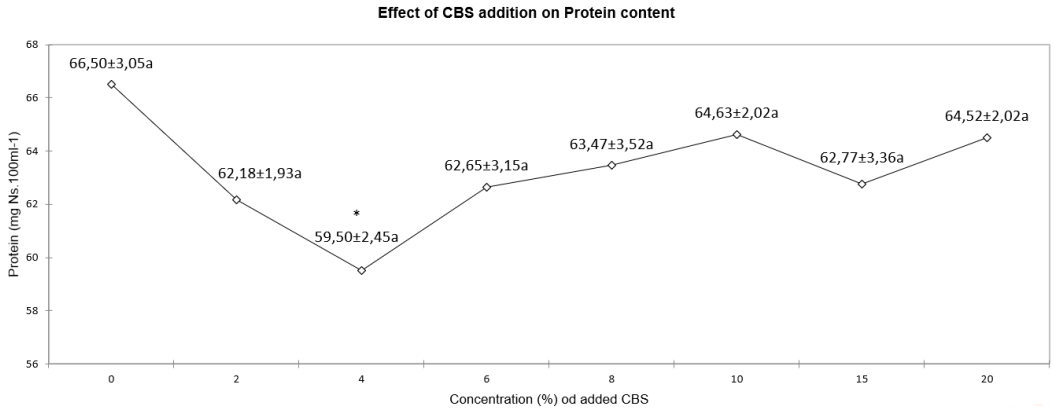

Figure 6 Effect of CBS addition on Nitrogen/Protein content.

CONCLUSION

In conclusion, we recorded a significant impact in five of the six parameters we tested. Only in protein content results were not significant. In traditional wort quality parameters, we recorded deterioration of these parameters. So, in a hypothetical application in conventional beer production, we must calculate the decline of density and increase of viscosity. Similar data were obtained in other applications in food where qualitative parameters with CBS addition deteriorate as well **(Jozinović *et al.*, 2019; Park *et al.*, 2017)**. Experiment on protein content showed that CBS is not only rich in protein, now we can say that it is rich in water-soluble protein too and we can use a significant amount of protein from CBS in wort-based beverages without a significant impact on overall protein content. Color changes showed significant and consistent increases so we can talk about this parameter as a model parameter which confirms our test model.

In the field of bioactive compounds, we recorded a significant increase in antioxidant activity and total phenolic content. Total phenolic content increase with addition but in antioxidative activity, we recorded higher activity in malt samples with different % inclusion of CBS than in samples in pure CBS extract, so the same part of the bioactive compound maybe insoluble in pure powder, and they are created by degradation during mashing. Or there is an antagonistic or synergic effect among antioxidants in malt and CBS. The complex interactions between polyphenols in food make it challenging to predict their combined effect on antioxidant activity, and further research is needed to understand the underlying mechanisms.

Acknowledgments: This research was funded by the Operational Program Integrated Infrastructure within the project: Demand-driven research for the sustainable and innovative food, Drive4SIFood 313011V336, co-financed by the European Regional Development Fund (40%) and by the project 06-GASPU-2021 (30 %), and KEGA grant 018SPU-4/2024 (30%).

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