SPECTROPHOTOMETRIC DETERMINATION OF BETALAINS AND TOTAL PHENOLS IN ALBUMIN CHEESE ENRICHED WITH ORGANIC BEETROOT POWDER

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Abstract: The aim of this study is to determine the change in the content of betalains and total phenols in albumin cheese enriched with organic beetroot powder during storage for 7 days. Four samples of albumin cheese were produced: ACK - a control sample of albumin cheese from whey, and three samples of albumin cheese enriched with organic beetroot powder, AC1, AC2, and AC3, containing 2.5%, 5.0%, and 7.5% beetroot powder, respectively. The content of betalains and their change during storage in the albumin cheese samples and organic beetroot powder were determined spectrophotometrically. For the determination of total phenols, two different solvents for extraction (50% ethanol and 80% methanol) were used, and the impact of the solvents, as well as the storage time of the samples at a temperature of 4°C, was assessed. The content of betalains in the AC1 sample was statistically significantly lower ($p<0.05$) compared to the other samples with a higher percentage of beetroot powder. Additionally, regarding the storage days, a statistically significant ($p<0.05$) reduction in the content of betalains was observed on the seventh day of storage. A statistically significant ($p<0.05$) increase in the content of phenols was determined in relation to the added percentage of beetroot powder, as well as a statistically significant ($p<0.05$) decrease in total phenols concerning the storage time. It was determined that a 50% ethanol solution has better extractive properties compared to an 80% methanol solution. The content of total phenols in the enriched albumin cheeses was higher ($p<0.05$) compared to the control sample ACK, indicating an improvement in the functionality of the newly formulated albumin functional cheeses.
Keywords: Functional albumin cheese, betalains, total phenols

1.INTRODUCTION

The initial use of beetroot (*Beta vulgaris* L. species) as an additive in food products was aimed at coloring and stabilizing the existing color. However, recent research opens the possibility of using beetroot as a functional ingredient (Fernández-López et al., 2023). The functionality of beetroot (Beta vulgaris L. species) is attributed to its numerous bioactive compounds, including phenols and betalains (Mudgal et al., 2022). Betalains are phenolic chromoalkaloids with health benefits for human health, demonstrating significant antioxidant, anti-inflammatory, antiviral and antitumor activities (Baião et al., 2020). Betalamic acid is a constituent of all betalains. The type of substituent on betalamic acid determines the class of betalains. Betacyanins (ranging from red to violet) contain a cyclo-3,4-dihydroxyphenylalanine (cyclo-DOPA) residue, whereas betaxanthins (yellow to orange) contain various amino acid or amine residues (Sadowska-Bartosz & Bartosz, 2021). Key representatives of betaxanthins include vulgaxanthin I, II, and indicaxanthin, while betacyanins include betanins, prebatanins, isobetanins, and neobetanins (Ravichandran et al., 2013). The most prevalent betacyanin in beetroot is betanidin 5-O-β-glucoside, which contains phenolic and cyclic amino groups that act as electron donors and serve as antioxidants (Kanner et al., 2001). Beetroot is the vegetable with the highest phenolic content (Mendelová et al., 2024), encompassing simple molecules (phenolic acids) to more complex polyphenols such as flavonoids (Arjeh et al., 2022). Phenolic compounds exhibit strong antioxidant properties and act as preventive agents against cancer, as well as reducing the risk of cardiovascular and chronic diseases (Guine et al., 2018).
Whey, as a byproduct of the dairy industry, is characterized by a high nutritional composition (Kalevska et al., 2018), and its processing into albumin cheese is one of the oldest methods of valorization (Bintsis & Papademas, 2023). However, the development of functional albumin cheeses has gained popularity in the last decade (Bintsis & Papademas,2023).

The aim of this research is to enrich albumin cheese (Urda) with varying percentages of organic beetroot powder, and to monitor the content of betalains and total phenols on the 1st and 7th day of storage. For the extraction of total phenols, two types of solvents, ethanol and methanol, were used to determine which solvent yields better extraction results.

2. MATERIALS AND METHODS

Reagents and Chemicals

Extraction was performed using 96% ethanol ("Alkaloid", Skopje, Macedonia) and methanol ("Carlo Erba", Germany). To prepare a 0.05M buffer solution with pH 6.5, disodium hydrogen phosphate dihydrate and dipotassium hydrogen phosphate trihydrate ("Alkaloid", Skopje, Macedonia), a 10% solution of sodium carbonate ("VWR", Canada), and Folin-Ciocalteau reagent ("MERCK", Germany) were used. For the spectrophotometric analyses, a 6715 UV/Vis spectrophotometer ("Jenway") was utilized. The analyses were conducted at the Faculty of Agriculture, University "Goce Delčev."

Production of Albumin Cheese

Albumin cheese (Urda) was produced solely from whey obtained during the production of cow's white brined cheese. Four samples of albumin cheese (Urda) were formulated: Control sample ACK – albumin cheese without the addition of beetroot powder, sample AC1 – albumin cheese enriched with 2.5% organic beetroot powder, sample AC2 – albumin cheese enriched with 5% organic beetroot powder, and sample AC3 – albumin cheese enriched with 7.5% organic beetroot powder. The organic beetroot powder used to enrich the albumin cheese samples was sourced from the producer "We are one", Serbia.

Determination of Betalains

The extraction of betalains from organic beetroot and enriched albumin cheese samples with beetroot powder was carried out following the method of Shakir & Simone (2024), with slight modifications. To 0.2 g of beetroot powder, 20 mL of 50% ethanol solution was added, or to 10 g of enriched albumin cheese, 30 mL of 50% ethanol solution was added. The samples were mixed and then centrifuged at 5000 rpm for 30 minutes. The determination of betalains was performed according to the method of (Giusti, 2001;Tumbas et al., 2016). The content of betacyanins was expressed as mg equivalents of betanin per 100 mL (mg BE/100 mL), and the content of betaxanthins was expressed as mg equivalents of vulgaxanthin-I per 100 mL (mg VE/100 mL). The total betalain content was calculated as the sum of betacyanins and betaxanthins.

Determination of Total Phenols

The extraction of total phenols from organic beetroot powder and albumin cheese was performed using two different solvents: 50% ethanol or 80% methanol.

0.2 g of beetroot powder and 20 mL 50% etanol was mixsed on a magnetic stirrer for 20 minutes, followed by centrifugation at 6000 rpm for 20 minutes. The centrifuged extract/supernatant was filtered and stored in a refrigerator at a temperature of up to 4ºC until analysis. For extraction using methanol as the solvent, the same procedure was followed, but instead of 20 mL of 50% ethanol, 20 mL of 80% methanol was used. For the extraction of total phenols from albumin cheese, the method described by Nakov et al., (2024) with slight modifications was applied. 10g of albumin cheese and 20 mL of 80% methanol was mixed for 20 minutes, and then centrifuged at 6000 rpm for 20 minutes and filtered. The filtered extract/supernatant was stored in a refrigerator at a temperature of up to 4ºC until analysis. For extraction using ethanol as the solvent, the same procedure was followed, but instead of 20 mL of 80% methanol, 20 mL of 50% ethanol was used.

The total phenols were analyzed according to the method of Ivanova et al., (2010) with slight modifications. In a 10 mL volumetric flask, 5 mL of distilled water, 1 mL of the prepared extract, and 0.5 mL of Folin-Ciocalteau reagent were added. The samples were allowed to stand for 3 minutes, after which 1.5 mL of 10% Na_2CO_3 was added, and the flask was filled to the mark with distilled water. The mixture was gently stirred and left to stand for 3 hours in the dark. The total phenol content was determined at a wavelength of 765 nm. The results were expressed as equivalents of gallic acid.

Statistical Analysis

For graphical representation of the results and determination of statistical differences, ANOVA (Analysis of Variance) and Fisher's LSD test for least significant differences with a 95% confidence factor ($p < 0.05$) were used, through the XLSTAT 2019 and Microsoft Office Excel 2019 software

3. RESULTS AND DISCUSSION

Table 1 presents the results of the analysis of betalain content as the sum of betacyanins and betaxanthins, in organic beetroot powder and the enriched samples with varying percentages of beetroot (AS1, AS2, and AS3) on the first and seventh days of storage.

In the organic beetroot powder, a betalain content of 9.64 mg/100 mL was determined. According to Farhan et al., (2024), the betalain content in beetroot powder was 2458.07 mg/100 g, while the content in sun-dried beetroot was 3.10 mg/g, and in freeze-dried beetroot, it was 4.89 mg/g (Bunkar et al., 2020). The betalain content in beetroot peel was 392.07 mg/L, with betacyanins accounting for approximately 60% of the betalains (236.53 mg/L) (Abdo et al., 2022).

Table 1. Betalain content in organic beetroot and albumin cheese samples

Sample		Betacyanins mg BE/100 mL	Betaxanthins mg BE/100 mL	Betalains mg/100 mL
Beetroot powder		5.57±0.02	4.40±0.33	9.64±0.93
AC1	day -1	4.61±0.02^{c,A}	2.48±0.01^{c,A}	7.09±0.01^{c,A}
	day -7	2.95±0.01^{c,B}	1.38±0.05^{c,B}	4.38±0.04^{c,B}
AC2	day -1	8.11±0.01^{b,A}	4.63±0.03^{b,A}	12.74±0.02^{b,A}
	day -7	6.73±0.01^{b,B}	4.06±0.24^{b,B}	10.82±0.29^{b,B}
AC3	day -1	11.83±0.02^{a,A}	6.37±0.05^{a,A}	18.16±0.08^{a,A}
	day -7	4.97±0,01^{a,B}	2.78±0.05^{a,B}	7.75±0.04^{a,B}

**Lowercase letters refer to statistically significant differences ($p < 0.05$) between samples with varying amounts of beetroot; uppercase letters refer to statistically significant differences ($p < 0.05$) between storage days.*

Source: Authors research

The content of betacyanins and betaxanthins in the AC1 sample was statistically significantly lower ($p < 0.05$) compared to the other samples AC2 and AC3, indicating that the betalain content in the enriched albumin cheese samples increases with the increase in the percentage of added organic beetroot powder. Additionally, regarding the storage days, a statistically significant ($p < 0.05$) decrease in the content of betacyanins was observed on the seventh day of storage, while the AC2 sample showed the best stability of betaxanthins. The largest decrease in betalain content between the first (18.16 mg/100 mL) and seventh (7.75 mg/100 mL) days of storage was observed in the AC3 sample, which contained the highest percentage of beetroot powder (7.5%).

The increase in betalain content relative to the control (whey protein drink) depending on the added percentage of beetroot peel extract, is also confirmed by Abdo et al., (2022). However, they observed instability of betalains during storage, with rapid degradation particularly after 7 days, with betalain contents reaching 62.6, 45.54, and 55.27 mg/L in the samples containing 1, 2.5, and 5% added beetroot extract, respectively. An increase in betalain content as a result of adding beetroot juice is also determined by Flores-Mancha et al., (2021), but in contrast to previous results, they show a statistically significant increase ($p < 0.05$) in betalain content from the 7th to the 14th day of storage, from 191.65 mg/100 g to 243.20 mg/100 g, respectively.

Table 2 presents the results of the analysis of total phenols in organic beetroot powder, the control sample, and the enriched albumin cheese samples using two solvents (50% ethanol and 80% methanol). From the table, it is observed that the organic beetroot powder contains 33.79 mg GAE/100 g when extracted with ethanol and 33.36 mg GAE/100 g when extracted with methanol. According to Abdo et al., (2022), the beetroot extract contains 21.70 mg GAE/g, while beetroot powder contained 12.18 mg GAE/g (Shalaby & Hassenin, 2020).

Regardless of the solvent used for extraction, higher values for phenolic compounds were observed in the enriched samples AC1, AC2, and AC3 compared to the control sample ACK. The total phenol content increased with the percentage of added beetroot powder on the first day of storage, reaching 25.19 mg GAE/100 g, 36.56 mg GAE/100 g, and 56.87 mg GAE/100 g for AC1, AC2, and AC3, respectively (when using 50% ethanol as the solvent). During storage, from the first to the seventh day, a decrease ($p < 0.05$) in total phenols was observed in the enriched samples AC1, AC2, and AC3. The greatest decrease was observed in the AC3 sample, while the least loss was noted in AC2 with 5% added organic beetroot powder, indicating the best stability. Nevertheless, in the enriched samples AC1, AC2, and AC3, regardless of the added percentage and storage time, higher total phenol values were determined compared to ACK, indicating an improvement in the functionality of the newly formulated functional albumin cheeses.

The total phenols in the whey protein drink enriched with 1%, 2.5%, and 5% beet peel extract increased compared to the control on the 0th day of storage, particularly for the samples with 2.5% and 5% added extract, which amounted to 36.12 and 43.21 mg/g, respectively (Abdo et al., 2022). Similarly, Flores-Mancha et al., (2021) observed an increase in phenols in yogurt with added beetroot juice during storage on the 1st, 7th, and 14th days, with values of 9.237 mg GAE/g, 9.262 mg GAE/g, and 9.795 mg GAE/g, respectively.

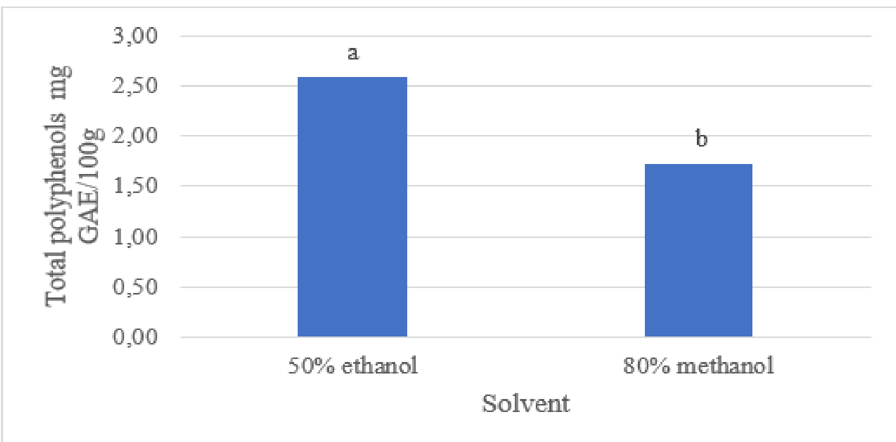
Table 2. Total phenol content in organic beetroot and albumin cheese samples

TOTAL PHENOLS mg GAE/100 g			
Sample	Analysis day	50% ethanol	80% methanol
Beetroot powder	/	33.79±0.10	33.36±0.12
ACK	day -1	7.52±0.02^{d,A}	2.77±0^{d,A}
	day -7	7.52±0.02^{d,B}	2.23±0.03^{d,B}
AC1	day -1	25.19±0.04^{c,A}	21.46±0.01^{c,A}
	day-7	15.65±0.02^{c,B}	14.66±0.02^{c,B}
AC2	day -1	36.56±0.06^{b,A}	29.26±0.08^{b,A}
	day-7	31.80±0.52^{b,B}	27.75±0^{b,B}
AC3	day-1	56.87±0.09^{a,A}	39.44±0.07^{a,A}
	day-7	25.95±0.02^{a,B}	19.33±0.04^{a,B}

**Lowercase letters refer to statistically significant differences ($p < 0.05$) between samples with different amounts of beetroot; uppercase letters refer to statistically significant differences ($p < 0.05$) between storage days.*
Source: Authors research

Figure 1 presents the average values of the total phenol content in albumin cheese samples extracted with 50% ethanol and 80% methanol.

Figure 1. Average value of total phenols, extracted with different solvents.



**Values with different letters are statistically significantly different ($p < 0.05$).*
Source: Authors research

A statistically significant difference ($p < 0.05$) was found in the phenol content when using 50% ethanol and 80% methanol as solvents. The total phenol content was higher when 50% ethanol was used as the solvent (2.59 mg GAE/100 g) compared to the total phenol content (1.73 mg GAE/100 g) when 80% methanol was used. According to Nouairi et al. (2021), 50% ethanol has been shown to be the solvent with the highest yield for betalain extraction. Due to the presence of phenolic and cyclic amino groups in the betalains from beetroot (Kanner et al., 2001), it is expected to obtain a better yield of phenols when using 50% ethanol, including the phenolic groups from betalains, compared to methanol as the solvent.

4.CONCLUSION

Fortifying albumin cheese with organic beetroot powder contributes to an increase in the content of betacyanins and betaxanthins, and thereby the total betalaine content. The betalaine content in sample AC1 is statistically significantly lower ($p < 0.05$) compared to AC2 and AC3. Additionally, regarding the storage days, a decrease ($p < 0.05$) in the content of betacyanins was observed on the seventh day of storage. Moreover, a higher content ($p < 0.05$) of total phenolic compounds was evident in the albumin cheese samples AC1, AC2, and AC3 compared to the control sample ACK on the first day of storage. Although a decrease ($p < 0.05$) in total phenols was observed during storage, all albumin cheese samples enriched with organic beetroot powder showed a higher content of total phenols, indicating an increase in the functionality of the newly formulated albumin cheeses. Regarding the solvents for phenolic compound extraction, ethanol demonstrated a better yield of phenols compared to methanol.

REFERENCES

Abdo, E. M., Allam, M. G., Gomaa, M. A., Shaltout, O. E., & Mansour, H. M. (2022). Valorization of whey proteins and beetroot peels to develop a functional beverage high in proteins and antioxidants. *Frontiers in Nutrition*, 9, 984891. https://doi.org/10.3389/fnut.2022.984891

Arjeh, E., Khodaei, S. M., Barzegar, M., Pirsa, S., Karimi Sani, I., Rahati, S., & Mohammadi, F. (2022). Phenolic compounds of sugar beet (*Beta vulgaris* L.): Separation method, chemical characterization, and biological properties. *Food science & nutrition*, *10*(12), 4238–4246. https://doi.org/10.1002/fsn3.3017

Baião, dos S. D., da Silva, D. V., & Paschoalin, V. M. (2020). Beetroot, a remarkable vegetable: Its nitrate and phytochemical contents can be adjusted in novel formulations to benefit health and support cardiovascular disease therapies. *Antioxidants*, *9*(10), 960. https://doi.org/10.3390/antiox9100960

Bintsis, T., & Papademas, P. (2023). Sustainable approaches in whey cheese production: A review. *Dairy*, 4(2), 249-270. https://doi.org/10.3390/dairy4020018

Bunkar, D. S., Anand, A., Kumar, K., Meena, M., Goyal, S. K., & Paswan, V. K. (2020).Development of production technology for preparation of beetroot powder using different drying methods. *Annals of Phytomedicine, 9(2),* 293-301. DOI: 10.21276/ap.2020.9.2.29

Farhan, M., Ahmad, Z., Waseem, M., Mehmood, T., Javed, M. R., Ali, M., & Goksen, G.(2024). Assessment of Beetroot powder as nutritional, antioxidant, and sensory evaluation in candies. *Journal of Agriculture and Food Research, 15*, 101023. https://doi.org/10.1016/j.jafr.2024.101023

Flores-Mancha, M. A., Ruíz-Gutiérrez, M. G., Sánchez-Vega, R., Santellano-Estrada, E., & Chávez-Martínez, A. (2021). Effect of encapsulated beet extracts (Beta vulgaris) added to yogurt on the physicochemical characteristics and antioxidant activity. *Molecules*, *26*(16), 4768. https://doi.org/10.3390/molecules26164768

Giusti, M. M. (2001). Current protocols in food analytical chemistry, F3.1.1-F3.1.7 Copyright © 2001 by John Wiley & Sons, Inc. DOI: 10.1002/0471142913.faf0402s01

Guine, R. P., Goncalves, F., Lerat, C., El Idrissi, T., Rodrigo, E., Correia, P. M., & Goncalves, J. C. (2018). Extraction of phenolic compounds with antioxidant activity from beetroot (*Beta vulgaris* L.). *Current Nutrition & Food Science*, *14*(4), 350-357. DOI: 10.2174/1573401313666170609102336

Ivanova, V., Stefova, M., & Chinnici, F. (2010). Determination of the polyphenol contents in Macedonian grapes and wines by standardized spectrophotometric methods. *Journal of the Serbian Chemical Society*, *75*(1), 45-59. DOI: 10.2298/JSC1001045I

Kalevska, T., Uzunoska, Z., Stamatovska, V., & Saveski, A. (2018). Whey properties and its use for production of functional and probiotic drinks. *Applied Researches in Technics, Technologies and Education, 6(1)* 50- doi: 10.15547/artte.2018.01.008

Kanner, J., Harel, S., & Granit, R. (2001). Betalains a new class of dietary cationized antioxidants. *Journal of Agricultural and Food chemistry*, *49*(11), 5178-5185. DOI: 10.1021/jf010456f

Mendelová, A., Mendel, Ľ., Solgajová, M., Kolesárová, A., & Mareček, J. (2024). Increasing the stability of polyphenols and betacyanins in beetroot chips by treatment before drying. *Journal of microbiology, biotechnology and food sciences*, *14*(1), e10936-e10936. https://doi.org/10.55251/jmbfs.10936

Mudgal, D., Singh, S., & Singh, B. R. (2022). Nutritional composition and value added products of beetroot: A review. *Journal of Current Research in Food Science*, *3*(1), 01-09.

Nakov, G., Trajkovska, B., Zlatev, Z., Jukić, M., & Lukinac, J. (2023). Quality characteristics of probiotic yoghurt enriched with honey and by-products left after the production of hemp oil by cold pressing the seeds of *Cannabis sativa* L. *Mljekarstvo: časopis za unaprjeđenje proizvodnje i prerade mlijeka, 73(1),* 3-11. https://doi.org/10.15567/mljekarstvo.2023.0101

Nouairi, M. E. A., Freha, M., & Bellil, A. (2021). Study by absorption and emission spectrophotometry of the efficiency of the binary mixture (Ethanol-Water) on the extraction of betanin from red beetroot. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *260*, 119939. DOI: 10.1016/j.saa.2021.119939

Ravichandran, K., Saw, N. M. M. T., Mohdaly, A. A., Gabr, A. M., Kastell, A., Riedel, H.,Cai, Z., Knorr, D., & Smetanska, I. (2013). Impact of processing of red beet on betalain content and antioxidant activity. *Food research international*, *50*(2), 670-675. https://doi.org/10.1016/j.foodres.2011.07.002

Sadowska-Bartosz, I., & Bartosz, G. (2021). Biological properties and applications of betalains. *Molecules*, *26*(9), 2520. https://doi.org/10.3390/molecules26092520

Shakir, B. K., & Simone, V. (2024). Estimation of betalain content in beetroot peel powder. *Italian Journal of Food Science*, *36*(1), 53-57. https://doi.org/10.15586/ijfs.v36i1.2438

Shalaby, H. S., & Hassenin, A. S. (2020). Effects of Fortification stirred yoghurt with red beetpowder (RBP) on Hypercholesterolemia Rats. *European Journal of Agriculture and Food Sciences, 2*(5). DOI: http://dx.doi.org/10.24018/ejfood.2020.2.5.61

Tumbas Šaponjac, V., Čanadanović-Brunet, J., Ćetković, G., Jakišić, M., Djilas, S., Vulić, J., & Stajčić, S. (2016). Encapsulation of beetroot pomace extract: RSM optimization, storage and gastrointestinal stability. *Molecules*, *21*(5), 584. https://doi.org/10.3390/molecules21050584