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Mass transfer rate and osmotic treatment efficiency of peaches

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Abstract. The highest-quality peaches [*Prunus persica* (L.) Batsch] are cultivated in areas with sunny summers, therefore the territory of the Autonomous Province of Vojvodina is a favourable region for their production. Peaches are usually consumed fresh, canned, or dried and represent a great source of the essential nutrients. Osmotic dehydration is a well-known preservation method that relies on mild temperatures and requires low energy. Research conducted at the Faculty of Technology Novi Sad has introduced sugar beet molasses as an efficient osmotic solution for drying various food samples. In this research, peach samples were osmotically treated in sugar beet molasses, and the goal was to investigate the impact of different solution concentrations, temperatures, and immersion time on the mass transfer rate and the efficiency of treatment. The results have shown that the mass transfer rate during the osmotic treatment of peach samples in sugar beet molasses was the most intensive at the beginning of the process, at the highest solution concertation, and at the highest temperature. In accordance with the results, diffusion occurred most rapidly during the first three hours of the process; therefore, processing time can be reduced.

Keywords and phrases: osmotic drying, sugar beet molasses, weight reduction, dehydration efficiency index, PCA, correlations

1. Introduction

Peaches are aromatic fruits with a specific, enjoyable, sweet taste, high organoleptic properties, nutritional values, and many commercial varieties (*Mihaylova et al.*, 2022; *Veerappan et al.*, 2021; *Lin et al.*, 2020). The Autonomous Province of Vojvodina is an advantageous region for the production of peaches due to good climate conditions (*Bulatović et al.*, 2017). Peaches are highly perishable and need preservation techniques to improve their shelf life (*Ayub et al.*, 2021).

Numerous processing methods can be used to preserve fruits; however, drying is the oldest form of food preservation, mainly because of water removal from the food samples, which slows down the action of the microorganisms (*Najafi et al.*, 2014; *Chavan & Amarowicz*, 2012). Osmotic dehydration, as one of the popular drying methods, attracts attention due to enhanced drying efficiency and reduced drying time (*Kutlu*, 2021; *Sakooei-Vayghan et al.*, 2020). Osmotic treatment involves preserving the freshness of plant and animal samples by immersion in an osmotic solution, enabling the flow of water molecules from the samples to an osmotic medium, and, to a lower extent, it transfers solutes from the solution into the samples, resulting in intermediate moisture products with acceptable organoleptic properties containing lower water activity, solute gain, and water loss (*Ramya & Jain*, 2017; *Ahmed et al.*, 2016). The gain of solids from the solution by the food leads to enriched foods by incorporating in the food matrix the compounds from the osmotic solution; for this reason, the selection of the proper hypertonic medium is crucial for the quality improvements of the dried product (*Abrahão & Corrêa*, 2021; *Shete et al.*, 2018).

Sugar beet molasses, a side-product of the sugar industry, has been confirmed as a satisfactory osmotic medium due to its technological effectiveness in water removal and its rich nutritional composition and low cost (*Nićetin et al.*, 2021a).

This investigation aimed to examine the effect of various osmotic solution concentrations, temperatures, and immersion time on the mass transfer rate (rate of water loss – RWL, rate of solid gain – RSG, and rate of weight reduction – RWR) and the efficiency of osmotic treatment of domestic peach samples in sugar beet molasses (weight reduction – WR – and dehydration efficiency index – DEI).

2. Materials and methods

Fruit material and osmotic solution

Fresh peaches (*Prunus persica*, var. *nucipersica*) were bought at the local market, with the initial dry matter content of fruit pulp: 7.40 ± 0.08%, were prepared by washing with running water, drying with paper towels, and peeling and cutting into cubes of about 1 × 1 × 1 cm.

Sugar beet molasses, obtained from the sugar factory Crvenka, Serbia, with a dry matter content of 85.04%, was used to prepare osmotic solutions. Sugar beet molasses was diluted to the concentrations of 60%, 70%, and 80% of dry matter by distilled water to prepare osmotic solutions.

Osmotic treatment

The osmotic dehydration treatment was conducted in laboratory jars, under atmospheric pressure, at a constant temperature of 20, 35, or 50°C, in a thermostat chamber (Memmert IN160, Germany) for 1, 3, and 5 hours respectively. Nine laboratory jars were used (three for each temperature); in each of them, 50 g of peaches were immersed in sugar beet molasses solution using a mash lid. The peach samples to molasses solution weight ratio were 1:5. After immersion in molasses, peach samples were stirred manually every 15 minutes to enhance the diffusion of the leaked water from the peach surface into the molasses. After the treatment time (1 h, 3 hrs, and 5 hrs), samples were taken out from the molasses solutions, quickly washed with water stream, and gently blotted to remove the excess of water. The dry matter content of osmotically dehydrated samples was determined by drying at 105°C for 24 hrs in a heat chamber (Instrumentaria Sutjeska, Croatia) until reaching constant weight. All analytical measurements were carried out following AOAC (2000).

Calculations

Calculations of osmotic parameters (rate of water loss – RWL, rate of solid gain – RSG, weight reduction – WR, rate of weight reduction – RWR) during the osmotic treatment of peach were performed as described by *Koprivica et al.* (2010).

Statistical analysis

The principal component analysis (PCA) has been applied effectively to classify and segregate the different samples. The analysis of variance (ANOVA) and PCA were performed using StatSoft Statistical software v.10 (Stat soft Inc., Tulsa, OK, USA). R Studio 1.4.1106 program was used for colour correlation graph between the obtained mass transfer rate parameters, the WR and DEI of peach samples.

3. Results and discussions

Table 1 displays the average values and standard deviations of the mass transfer rate, weight reduction, and dehydration index during the osmotic treatment of peach samples as a function of immersion time, osmotic solution concentration,

and temperature. Based on the obtained results, osmotic treatment was the most intensive initially. Also, it can be noticed that higher values of mass transfer rate were obtained at higher concentrations of sugar beet molasses solution and a higher temperature.

ANOVA showed that for RWL, RSG, RWR, WR, and DEI values, there was a significant statistical difference between the values of the peach samples osmotically treated for 1, 3, and 5 hours. In addition, there is a significant statistical difference between the values of the peach samples osmotically treated at different molasses solution concentrations (60%, 70%, and 80% w/w) and temperatures (20, 35, and 50°C).

Table 1. Mass transfer rate, weight reduction, and dehydration index during the osmotic treatment of peaches

No.	t (h)	C (% w/w)	T (°C)	RWL $g/(g_{i.s.w.}\cdot s)\cdot 10^5$	RSG $g/(g_{i.s.w.}\cdot s)\cdot 10^5$	RWR $g/(g_{i.s.w.}\cdot s)\cdot 10^5$	WR $g/g_{i.s.w}$	DEI
1	1	60	20	8.79 ± 0.04^{l}	0.53 ± 0.01^{f}	8.25 ± 0.10^{k}	0.30 ± 0.002^{a}	8.84 ± 0.17^{p}
2	3	60	20	4.50 ± 0.04^{f}	0.43 ± 0.07^{c}	4.12 ± 0.03^{e}	0.44 ± 0.003^{f}	5.48 ± 0.11^{hi}
3	5	60	20	3.29 ± 0.02^{a}	0.32 ± 0.02^{a}	2.97 ± 0.03^{a}	0.54 ± 0.001^{k}	5.41 ± 0.04^{gh}
4	1	70	20	9.78 ± 0.01^{m}	0.84 ± 0.01^{l}	8.71 ± 0.01^{l}	0.31 ± 0.002^{b}	6.45 ± 0.04^{lm}
5	3	70	20	4.81 ± 0.09^{g}	0.39 ± 0.01^{b}	4.41 ± 0.09^{f}	0.47 ± 0.004^{g}	6.49 ± 0.09^{n}
6	5	70	20	3.40 ± 0.06^{ab}	0.32 ± 0.02^{a}	3.08 ± 0.03^{a}	0.55 ± 0.002^{lm}	5.56 ± 0.05^{ij}
7	1	80	20	11.67 ± 0.06^{p}	1.06 ± 0.01^{n}	10.61 ± 0.05^{o}	0.38 ± 0.001^{d}	5.94 ± 0.06^{jk}
8	3	80	20	5.18 ± 0.03^{h}	0.47 ± 0.01^{l}	4.72 ± 0.12^{g}	0.51 ± 0.004^{hi}	5.13 ± 0.05^{kl}
9	5	80	20	3.81 ± 0.04^{cd}	0.39 ± 0.04^{b}	3.44 ± 0.01^{cd}	0.61 ± 0.005^{op}	4.82 ± 0.05^{f}
10	1	60	35	9.92 ± 0.01^{m}	0.84 ± 0.02^{l}	8.95 ± 0.01^{l}	0.32 ± 0.001^{b}	5.51 ± 0.12^{m}
11	3	60	35	4.98 ± 0.10^{gh}	0.38 ± 0.01^{b}	4.60 ± 0.04^{fg}	0.49 ± 0.005^{h}	6.08 ± 0.22^{o}
12	5	60	35	3.44 ± 0.05^{ab}	0.32 ± 0.03^{a}	3.11 ± 0.03^{ab}	0.56 ± 0.005^{mn}	5.77 ± 0.10^{ij}
13	1	70	35	10.43 ± 0.04^{n}	0.94 ± 0.01^{m}	9.43 ± 0.01^{m}	0.34 ± 0.001^{c}	5.19 ± 0.12^{kl}
14	3	70	35	5.57 ± 0.08^{i}	0.48 ± 0.02^{e}	5.00 ± 0.07^{h}	0.54 ± 0.001^{kl}	5.52 ± 0.10^{k}
15	5	70	35	3.86 ± 0.07^{d}	0.35 ± 0.04^{l}	3.56 ± 0.02^{cd}	0.64 ± 0.002^{q}	5.31 ± 0.11^{lm}
16	1	80	35	11.05 ± 0.07^{o}	1.03 ± 0.02^{n}	10.12 ± 0.03^{n}	0.36 ± 0.003^{d}	4.83 ± 0.07^{ij}
17	3	80	35	5.70 ± 0.05^{i}	0.50 ± 0.04^{e}	5.23 ± 0.02^{hi}	0.56 ± 0.007^{lm}	5.57 ± 0.04^{lm}
18	5	80	35	3.95 ± 0.05^{de}	0.39 ± 0.01^{b}	3.64 ± 0.03^{d}	0.64 ± 0.001^{q}	5.29 ± 0.19^{g}
19	1	60	50	11.34 ± 0.02^{op}	1.18 ± 0.02^{l}	10.05 ± 0.01^{n}	0.36 ± 0.004^{d}	5.55 ± 0.060^{f}

No.	t (h)	C (% w/w)	T (°C)	RWL $g/(g_{i.s.w.} \cdot s) \cdot 10^5$	RSG $g/(g_{i.s.w.} \cdot s) \cdot 10^5$	RWR $g/(g_{i.s.w.} \cdot s) \cdot 10^5$	WR $g/g_{i.s.w}$	DEI
20	3	60	50	5.43 ± 0.06^{i}	0.67 ± 0.01^{l}	4.84 ± 0.06^{g}	0.52 ± 0.002^{ij}	4.31 ± 0.06^{d}
21	5	60	50	3.59 ± 0.05^{bc}	0.53 ± 0.04^{l}	3.10 ± 0.08^{a}	0.56 ± 0.005^{lm}	3.92 ± 0.06^{a}
22	1	70	50	13.08 ± 0.01^{q}	1.49 ± 0.02^{l}	11.40 ± 0.01^{p}	0.42 ± 0.001^{e}	4.73 ± 0.08^{e}
23	3	70	50	6.23 ± 0.16^{j}	0.79 ± 0.01^{l}	5.37 ± 0.05^{i}	0.58 ± 0.005^{n}	4.78 ± 0.07^{c}
24	5	70	50	3.87 ± 0.05^{d}	0.55 ± 0.01^{l}	3.38 ± 0.03^{bc}	0.61 ± 0.007^{o}	3.95 ± 0.03^{ab}
25	1	80	50	13.33 ± 0.02^{r}	1.85 ± 0.04^{l}	11.51 ± 0.02^{p}	0.41 ± 0.004^{e}	3.20 ± 0.05^{b}
26	3	80	50	6.52 ± 0.03^{k}	0.72 ± 0.02^{l}	5.69 ± 0.17^{j}	0.62 ± 0.003^{p}	5.01 ± 0.07^{e}
27	5	80	50	4.23 ± 0.05^{e}	0.60 ± 0.01^{l}	3.63 ± 0.03^{cd}	0.65 ± 0.006^{q}	3.03 ± 0.01^{ab}

Note: $^{a\text{-}r}$ – the different letters in the superscript of the datasets regarding peach samples indicate a statistically significant difference between values, at a level of significance of $p < 0.05$.

Water loss rates, solid gain rates, and mass reduction rates showed the highest values during the first hour of the osmotic treatment. As previously noticed in other research (*González-Pérez et al.*, 2021; *Prithani & Dash*, 2020; *Assis et al.*, 2016; *Nićetin et al.*, 2014), the mass transfer rate decreased continuously from the first to the third hour, and after the third hour it showed a tendency of slowing down. The mass transfer rate was intensive when peach samples were osmotically treated in the most concentrated solution and at the highest temperature because of a more significant difference between the osmotic pressures of the hypertonic medium and the peach samples' tissue. The highest values for RWL, RSG, and RWR (13.33 ± 0.02, 1.85 ± 0.04, and 11.51 ± 0.02 respectively) were obtained after 1 h of osmotic treatment in a sugar beet molasses solution of 80% w/w concentration and with a temperature of 50 °C.

Weight reduction (WR) occurred due to water transfer from the peach sample in the osmotic medium and the smaller extent diluted nutrients from the sugar beet molasses solution into the immersed peach pieces. Osmotic treatment results in the reduced mass of the samples as a consequence; the food samples lose weight and shrink (*Chandra & Kumari*, 2015). From *Table 1*, it can be seen that the highest value of WR was reached after 5 hrs; using 80% sugar beet molasses solution at 50°C, the WR value was 0.65 g/g of the initial sample weight. The sample mass was reduced the most rapidly in the first three hours of the process.

The value of DEI is the most crucial criterion of the efficiency of the osmotic treatment (*Ćurčić et al.*, 2014). Considering the fact that DEI represents a ratio of water loss and solid gain during the treatment, a higher concentration of the osmotic medium tends towards the diffusion of solids into the sample, which results in a

reduction in the value of DEI. The highest value of DEI was noticed at the beginning of the process: 8.84 ± 0.17. The tendency of DEI decrease could be explained by solid gain increase of peach from the molasses during the osmotic treatment process. This phenomenon had a positive effect on the chemical composition of dehydrated samples, considering the rich nutritional composition of sugar beet molasses (*Lončar et al.*, 2021; *Nićetin et al.*, 2021).

The obtained correlations were illustrated in *Figure 1*, employing the "corrplot" function from the R Studio 1.4.1106 program. The colour is based on the correlation coefficients and indicates a relation between two samples; if the colour is blue, the positive correlation was accomplished; on the contrary, the red colour symbolizes negative correlation between the samples. The darker blue tone of the squares suggests a stronger correlation between these samples, while the lighter tone indicates an evident dissimilarity between the samples.

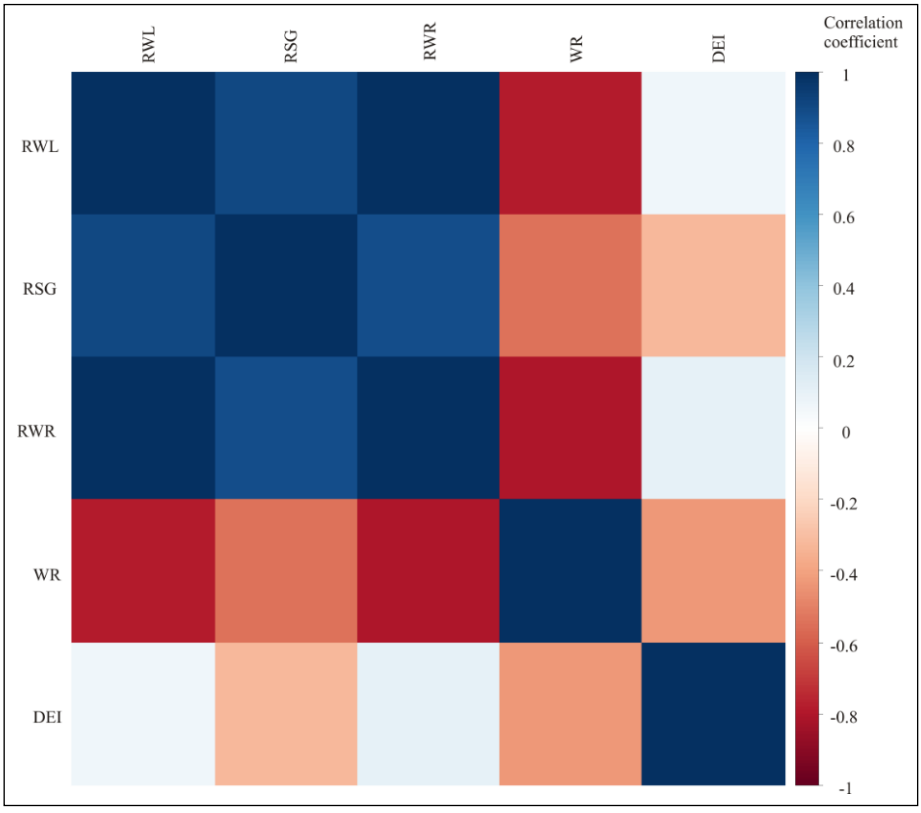

Figure 1. Colour correlation graph between obtained mass transfer rate parameters, WR and DEI of peach samples

From *Figure 1*, we can observe a positive correlation between RWL, RSG, and RWR and a negative correlation of these responses to WR.

In order to better explain the structure of the exploratory data that would contribute to the comprehension of likenesses and dissimilarities of the peach samples, PCA was applied, and the results are presented in *Figure 2*. The PCA of the mass transfer rate parameters, WR and DEI of the peach samples explained that the first two principal components summarized 81.93% of the total variance in the observed parameters. The projection of the factors indicated that RWL, RSG, RWR, and WR contributed mostly to the first principal component PC1 (28.25%, 22.58%, 28.28%, and 20.60% respectively), while the DEI contributed more to the second principal component PC2 (71.93%).

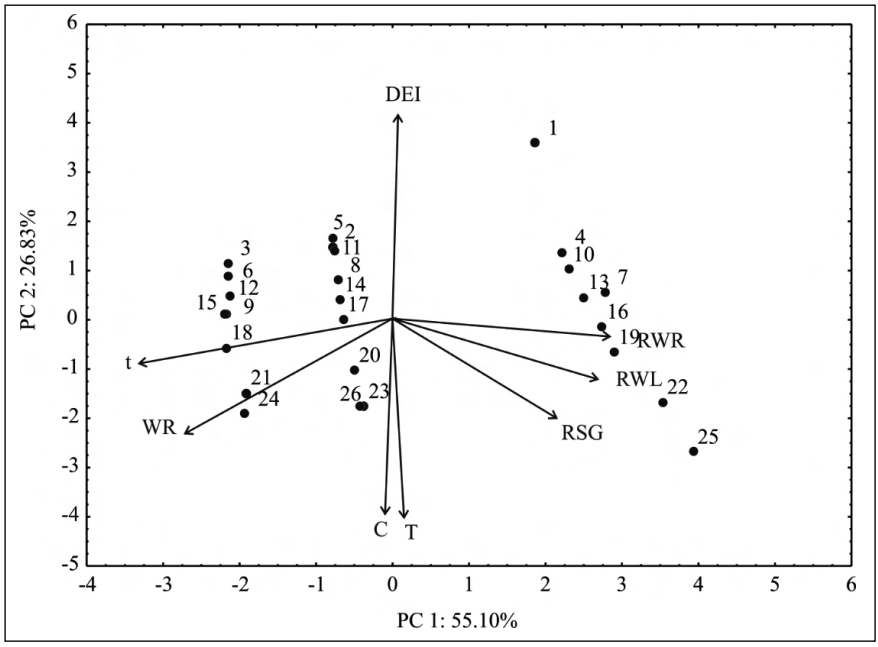

Figure 2. The PCA biplot diagram describing the relations between mass transfer rate parameters, WR and DEI of peach samples (the samples' codes are noted in *Table 1*)

4. Conclusions

Based on the given results, it can be concluded that sugar beet molasses is an effective osmotic solution for the osmotic treatment of peach samples. During the osmotic treatment of peach samples, the water-removing process was the most intensive at the beginning in all osmotic solutions. After 3 hours, it tended to

slow down; therefore, 3 hours could be set as the processing time for the osmotic treatment of the peach. Osmotic dehydration provides a semi-product, and the desired final product is obtained in the second drying step using other dying methods. The PCA of the mass transfer rate parameters, weight reduction, and dehydration efficiency index of peach samples explained that the first two principal components summarized 81.93% of the total variance in the observed parameters.

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The combined effect of time and temperature during oven drying on red grape pomace polyphenols, pigments, and antioxidant properties

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Abstract. This study had as a goal to carry out the drying of red grape pomace (RGP) using a 2 × 3 factorial design. The design of the experiment included combinations of time and temperature in order to achieve the lowest possible moisture levels and examine losses in precious polyphenols, but also the effect on the antioxidant properties of RGP extracts. Drying for 6 hrs at 80°C (D6/80) provided RGP with a satisfactory moisture level (11%). A comparison with untreated (fresh) RGP revealed that drying significantly decreases the polyphenol and anthocyanin pigments' content. This decline was accompanied by a decrease in both the ferric-reducing power and antiradical activity of the RGP extracts. Although necessary for long-term RGP stability, drying should be implemented with caution because improper drying may have severe effects on the polyphenolic composition and antioxidant activity.

Keywords and phrases: anthocyanins, antioxidants, drying, red grape pomace, polyphenols

1. Introduction

The winemaking industry is of paramount importance to the agricultural sector worldwide, as grapes are one of the primary fruit crops cultivated globally. Grape production was about 77.8 metric tons in 2018. Based on the International Organization of Vine and Wine (OIV) statistics, 292 million hectolitres of wine were produced worldwide in 2018 (*Ahmad et al.*, 2020). As a result, the wine industry regularly generates a vast amount of waste, posing serious environmental risks associated with the release of rejected biomass with high chemical and biological oxygen demand. On the other hand, this large pool of underutilized residual material is considered an outstanding bioresource of an array of compounds that could be valorized for the production of high value-added products.

Vinification wastes are mainly composed of vine shoots, grape pomace, and stems. The two latter side-streams are particularly rich in polyphenols, including several classes such as hydroxycinnamates, flavanols, flavonols, and anthocyanins. Many of these compounds may possess biologically important bioactivities, including antioxidant activity, antimicrobial action, anti-inflammatory properties, etc. (*Georgiev et al.*, 2014; *Teixeira et al.*, 2014). Such scientific evidence has led to the development of numerous valorization approaches for winemaking wastes, encompassing strategies that aimed at optimizing raw material handling and the optimization of polyphenol recovery, through improved solid-liquid extraction techniques (*Hogervorst et al.*, 2017; *Makris*, 2018).

One of the critical handling steps taken to prepare grape pomace for subsequent extraction is undisputedly the drying process (*Rajha et al.*, 2014; *Sui et al.*, 2014). Drying is commonly performed to increase preservation time, but it also facilitates pulverization, which is crucial for an effective extraction. The generation of a fine powder out of the dried plant material allows for high mass transfer during solid-liquid extraction and significantly increases extraction yield (*Sridhar et al.*, 2021). However, in procedures destined to acquire a properly prepared plant material for subsequent extraction, drying is performed rather empirically. Thus, its effect on the target substances is not thoroughly appraised. Improper drying may significantly deteriorate grape pomace, with detrimental consequences to the polyphenolic composition and antioxidant activity (*Marchante et al.*, 2018).

On this ground, the present investigation was performed to identify the most suitable conditions that would result in an effective grape pomace drying and to examine losses in precious polyphenolic compounds. To this purpose, several combinations of time and temperature were tested to identify the optimal set of conditions by recording changes in thermolabile polyphenol groups such as flavanols and anthocyanins. The impact on typical in vitro antioxidant properties was also evaluated to further assess the drying conditions used. The composition of the optimally dried material was investigated by carrying out liquid chromatography-mass spectrometry analyses.

2. Materials and methods

2.1 Chemicals and reagents

Solvents used for chromatographic analyses were of HPLC grade. The gallic acid hydrate was from Panreac (Barcelona, Spain). The L-Ascorbic acid (99.5%), quercetin, Folin-Ciocâlteu reagent, 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), rutin (quercetin 3-*O*-rutinoside) (> 94%), catechin, *p*-dimethylaminocinnamaldehyde (DMACA), and 2,2-diphenylpicrylhydrazyl (DPPH) were from Sigma-Aldrich (Darmstadt, Germany). Pelargonin (pelargonidin 3,5-di-*O*-glucoside) chloride was from Extrasynthese (Genay, France). Sodium carbonate anhydrous (99%) was from Penta (Praha, Czechia).

2.2 Red grape pomace (RGP)

RGP was obtained from the industrial vinification of *Vitis vinifera* cv. Muscat of Hamburg grapes, and it was provided by a winery located in Karditsa (central Greece). The collected material was transferred to the laboratory within 2 hrs after collection and stored at -40°C until used.

2.3 Determination of moisture content

RGP was thawed and placed on a tray in layers of approximately 0.5 cm thickness. The material was dried in a laboratory oven (Binder BD56, Bohemia, NY, USA) at 105°C for 48 hrs. The % moisture content was then determined as follows:

$$u\% = 100 \cdot \frac{f_m - d_m}{d_m} \quad (1)$$

The terms f_m and d_m correspond to fresh and dry mass (g), and u% is the moisture content in mass percentage.

2.4 Drying assay

The aim was to provide RGP with the lowest possible moisture level by appropriately combining the two critical drying variables, temperature (T) and time (t). In this framework, several combinations were tested by using T levels varying from 50 to 80°C and t processing time ranging from 3 to 9 hrs. After drying, RGP was ground in a ball mill to provide a powder with an average particle diameter of dp = 384 μm. The obtained powder was filled in airtight plastic vessels and stored in the dark at 4°C.

2.5 Extraction of RGP

An amount of 0.5 g of RGP powder was placed in a 50-mL round-bottom flask with 20 mL of 70% ethanol containing 0.1% HCl. The mixture was extracted under magnetic stirring at 500 rpm, for 180 min, and at 40°C by a thermostated hotplate (Witeg, Wertheim, Germany). After extraction, 1 mL of the sample was transferred in a 1.5 mL Eppendorf tube and centrifuged for 10 min at 10000 × g. The clear supernatant was used for all further analyses.

2.6 Design of experiment

The purpose was to assess the combined effect of drying time (t) and temperature, (T) (independent variables) on the residual moisture content, u (%) (response). These two variables were considered because of their profound influence on the drying of plant materials. To accomplish this, a 2 × 3 factorial design was implemented as previously described (*Khiary et al.*, 2009). This statistical approach was used to identify the relationship between the response function and process variables and to determine the optimal conditions for the drying process (minimization of the residual moisture content). The two independent variables, t and T, varied from 3 to 6 hrs and from 50 to 80°C respectively (*Table 1*). These variation ranges were chosen based on both preliminary experiments and bibliographic data. All experiments were performed in triplicate.

Table 1. Selected combinations of process variables of oven drying (experimental design)

Design point	t (h)	T (°C)
1	3	50
2	3	65
3	3	80
4	6	50
5	6	65
6	6	80

2.7 Total polyphenol (TP) determination

Samples were first diluted 1:50 with 0.5% (v/v) aqueous formic acid, and analysis was carried out according to a previously described micro-scale methodology (*Cicco et al.*, 2009). Briefly, 0.1 mL Folin-Ciocâlteu reagent and 0.1 mL of diluted

sample were mixed in a 1.5 mL Eppendorf tube and allowed to react for 2 min. Then 0.8 mL of Na_2CO_3 solution (5% w/v) was added, and the mixture was incubated in a water bath for 20 min, at 40°C. After incubation, samples were cooled down with tap water and measurement of the absorbance at λ = 740 nm was performed.

The total polyphenol concentration (C_{TP}) was determined from a calibration curve, using gallic acid as standard (10 – 80 mg·L^{-1}, R^2 = 0.9996). Results were given as mg gallic acid equivalents (GAE) L^{-1}. Yield in TP (YTP) was expressed as mg GAE g^{-1} dry mass (DM) (*Grigorakis et al.*, 2020).

2.8 Total monomeric anthocyanin (TA) determination

TA were estimated with the pH-differential method (*Lee et al.*, 2005). A volume of 0.1 mL RGP extract was combined with 0.9 mL KCl buffer (pH = 1.0), and the absorbance was obtained at both λ = 520 nm and λ = 700 nm. Similarly, RGP extract was also combined with CH_3COONa buffer (pH = 4.5), and the corresponding absorbances were read. The total anthocyanin concentration (C_{TA} expressed in mg·L^{-1}) was calculated as follows:

$$C_{TA} = \frac{A \cdot MW \cdot F_D \cdot 10^3}{\varepsilon} \quad (2)$$

A corresponds to $(A_{520} - A_{700})_{pH = 1} - (A_{520} - A_{700})_{pH = 4.5}$, and MW is the molecular weight of malvidin 3-*O*-glucoside (MW = 529 g·mol^{-1}). F_D is the dilution factor (1:10), and λ is the molar absorptivity of malvidin 3-*O*-glucoside (λ = 28,000). Results were given as malvidin 3-*O*-glucoside equivalents (MvE).

2.9 Total flavanol (TF) determination

The *p*-dimethylaminocinnamaldehyde (DMACA) assay (*Makris et al.*, 2008) was used, with some modifications. Samples were diluted with methanol (1:50), and an amount of 0.02 mL of diluted sample was transferred in a 1.5 mL Eppendorf tube, along with 0.1 mL DMACA (0.1% w/v in methanol) and 0.88 mL HCl (2 M in methanol). After exactly 15 min, absorbance readings were obtained at λ = 640 nm. Total flavanol concentration (C_{TF}) was determined from a catechin calibration curve (1 – 80 mg·L^{-1}, R^2 = 0.9999) and given as catechin equivalents (CtE).

2.10 Antioxidant properties

The antiradical activity (A_{AR}) determination was performed with a stoichiometric assay, using as chromophore probe the stable DPPH radical, as described elsewhere (*Chakroun et al.*, 2021). Results were given as μmol DPPH g^{-1} DM. Reducing

power (P_R) determination was carried out according to a previously published protocol (*Chakroun et al.*, 2021), and results were expressed as μmol ascorbic acid equivalents (AAE) g^{-1} DM.

2.11 Liquid chromatography-diode array-mass spectrometry (LC-DAD-MS)

The devices were a Finnigan (San Jose, CA, USA) MAT Spectra System P4000 pump, a Finnigan AQA mass spectrometer, and a UV6000LP diode array detector. Chromatography was performed on a Fortis RP-18 column (150 mm × 2.1 mm, 3 μm) at 40°C, with a 10 μL injection loop. Mass spectra acquisition was carried out with electrospray ionization (ESI) in both positive and negative ion mode. Details on mass spectrometry setting and elution have been described elsewhere (*Makris & Kefalas*, 2013).

2.12 High-performance liquid chromatography (HPLC) analysis

The equipment was a Shimadzu CBM-20A (Shimadzu Europa GmbH, Germany) coupled to a Shimadzu SPD-M20A detector and interfaced by a Shimadzu LC solution software. Chromatographic analyses were accomplished on a Phenomenex Luna C18(2) column (100 Å, 5 μm, 4.6 × 250 mm) (Phenomenex, Inc., Torrance, CA, USA), with the same guard column, at 40°C. Analytical details concerning the elution and solvents used have been previously reported (*Lakka et al.*, 2020). Quantification was carried out with the calibration curves of gallic acid (R^2 = 0.9990), caffeic acid (R^2 = 0.9980), catechin (R^2 = 0.9999), quercetin (R^2 = 0.9990), and pelargonin (R^2 = 0.9999). All calibration curves were constructed using methanolic solutions with concentrations in the range of 0–50 $\mu g \cdot mL^{-1}$.

2.13 Statistics

All extractions were accomplished at least twice, and all determinations were done in triplicate. The results were given as means with standard deviations. Distribution analysis was performed with JMP™ Pro 13 and linear regressions with SigmaPlot™ 12.5. At least a 95% significance level was applied to all statistical analyses.

3. Results and discussions

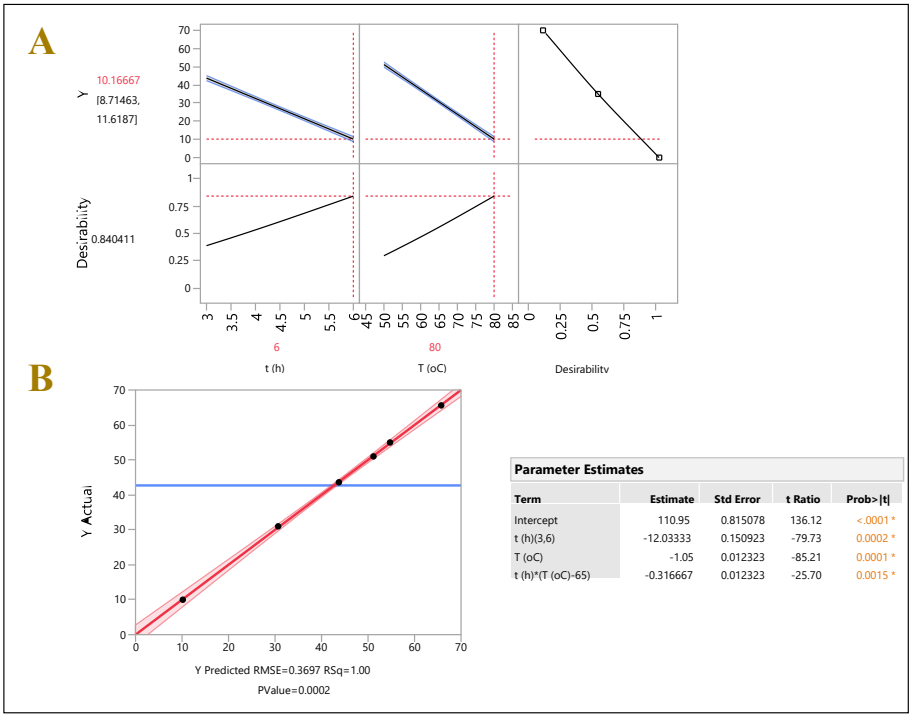
3.1 Drying optimization

The process was designed to assess the effect of two key drying variables, *t* and *T*, and to detect possible synergistic effects between them.

Table 2. The results of the drying experiments (in triplicate) in conformity with the chosen experimental design

***t* (h)**	***T* (°C)**	***u* (%)**
3	50	65.6 ± 3.2
3	65	55 ± 3.0
3	80	43.6 ± 2.7
6	50	51 ± 3.1
6	65	31 ± 2.1
6	80	10 ± 1.4

Assessment of the fitted model and response surface suitability was done by considering the closeness of the measured and predicted values (*Figure 1B*).



Notes: Graphs A and B correspond to the desirability function and actual-to-predicted diagram. Asterisk (*) in the inset table "Parameter Estimates" shows statistically significant terms, at least at a 95% significance level.

Figure 1. Model fitting and evaluation obtained by implementing a 2 × 3 factorial design.

The second-degree polynomial equation (mathematical model) derived was as follows:

$$u\% = 110.95 - 12.03 \cdot \left(\frac{t-4.5}{1.5}\right) + 0.33 \cdot T \cdot \left(\frac{t-4.5}{1.5}\right) \cdot (T - 65) \quad (3)$$

Since the total correlation coefficient of the model was $R^2 = 1.00$, and the $p = 0.0002$ (assuming a confidence interval of 95%) was highly significant, it could be supported that equation (3) represents a good fitting to the experimental data. The three-dimensional plot constructed on the basis of the model (*Figure 2*) is the graphical representation of residual moisture (response) dependence on the drying process parameters, the drying temperature (T), and the drying process duration (t).

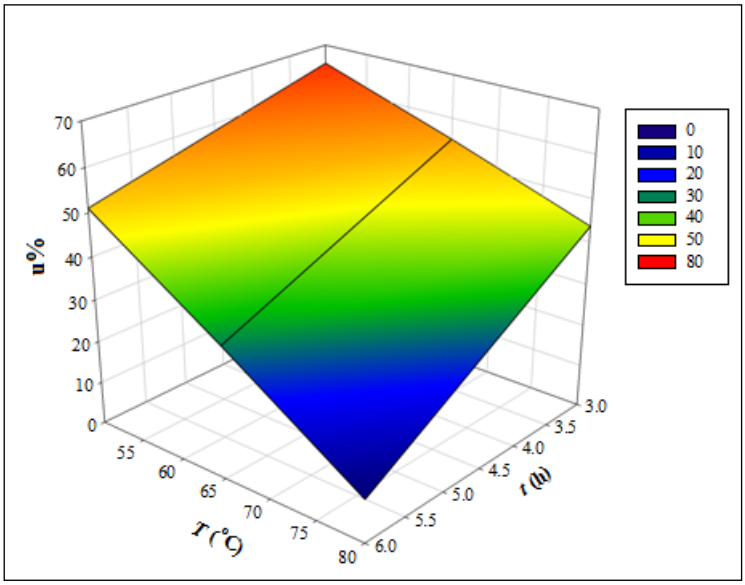


Figure 2. Effect of the drying process parameters (t and T) on residual moisture content (u)

Using the desirability function (*Figure 1A*), it could be estimated that to achieve a sufficiently low moisture level of around 10% (*Marchante et al.*, 2018), the values of the process parameters should be $t = 6$ h and $T = 80$°C. Under these conditions, the predicted moisture level was 10.2 ± 1.5%. This theoretical value was confirmed by performing three individual drying experiments under optimal conditions, where a value of 10.1 ± 2.0% was found. Thus, the process with these parameters ($t = 6$ h and $T = 80$°C), marked as D6/80, was considered for further investigation.

3.2 Effect of drying on polyphenols

As can be seen in *Figure 3*, D6/80 generated a decrease in total polyphenol content (Y_{TP}) by 18.3% compared with the fresh (non-dried) sample. Other studies on RGP drying showed that RGP oven-dried at 80°C for 24 hrs retained significantly more total polyphenols than when dried at 40°C for 72 hrs (*Demirkol & Tarakci*, 2018). Similarly, RGP dried at 60°C was found to better retain polyphenols compared with drying at either 40 or 50°C (*Teles et al.*, 2018). On the other hand, it has been reported that fluidized-bed drying of RGP at $T < 70$°C for 90 min was more effective in preserving polyphenolic content compared with 80°C for 180 min (*Planinić et al.*, 2015). In the same line, drying at $T < 60$°C for 3 days generated significantly less reduction in total polyphenols than at higher temperatures for 8 hrs (*Khanal et al.*, 2010). Furthermore, large increases in the drying temperature, from 60 to 140°C, have also been proven to be detrimental for total polyphenol retention (*Larrauri et al.*, 1997).

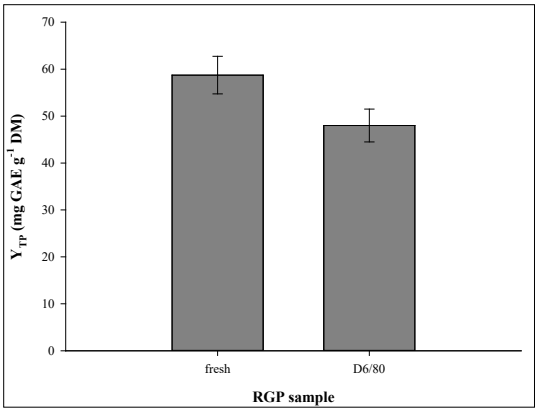


Figure 3. The effect of drying (D6/80) on the total polyphenol content (Y_{TP}) of the RGP in comparison with the fresh samples

To obtain a deeper insight into the effect of D6/80 drying on RGP polyphenols, two other indices were also considered: the yield in total flavanols (Y_{TF}) and the yield in total monomeric anthocyanins (Y_{TA}). These two major polyphenolic RGP constituents were selected according to their sensitivity to drying (*Çoklar & Akbulut*, 2017). Therefore, it would be expected that any destructive effect of drying could be clearly reflected on the changes in these indices. Indeed, it was a Y_{TF} decrease by 54.4% (*Figure 4A*); likewise, the reduction of Y_{TA} was 56.2% (*Figure 4B*).

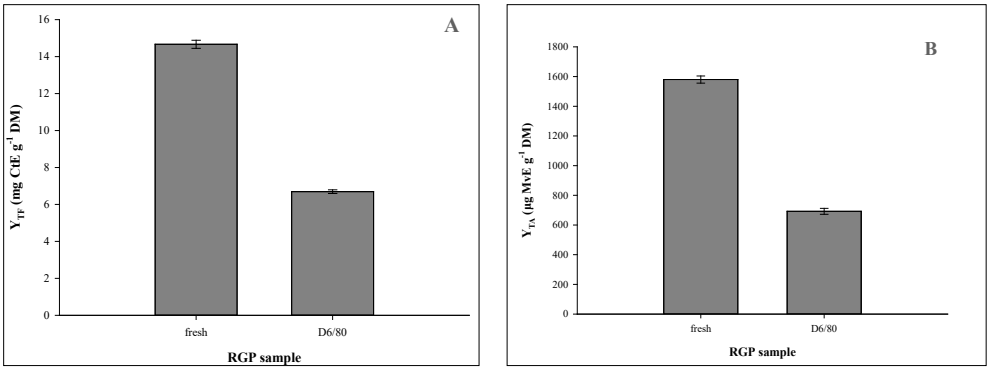
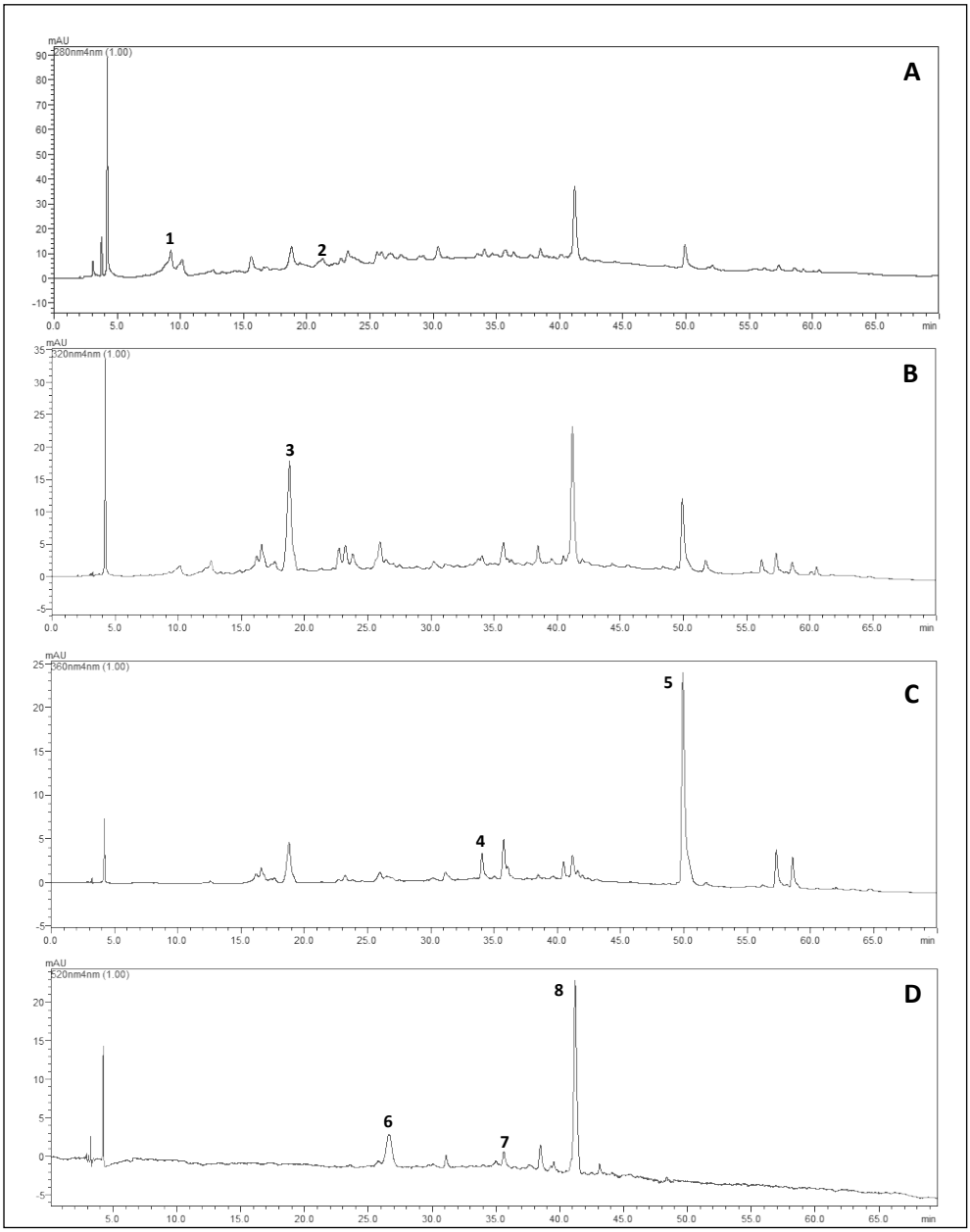


Figure 4. The effect of the drying processes (D6/80) on the yield of total flavanol (A) and total monomeric anthocyanin (B) of RGP

To obtain an integrated picture concerning the impact of drying on RGP polyphenols, the extract prepared after implementing D6/80 drying was analysed by HPLC and compared to the extract obtained from fresh (non-dried) sample. The analysis of both extracts revealed the presence of eight principal polyphenols (*Figure 5*), three of which were anthocyanin pigments. Catechin, rutin, gallic acid, and quercetin could be easily identified through a comparison of the corresponding retention times with those of commercial standards. Caftaric acid (caffeoyltartaric acid) was identified by its molecular ion at m/z = 311 using negative ion mode operation, based on previously published data (*Makris et al.*, 2003). Likewise, malvidin 3-*O*-glucoside was tentatively identified by its molecular ion at m/z = 493 and the diagnostic fragment at m/z = 331 (aglycone), in positive ion mode operation. Similarly, malvidin 3-*O*-glucoside acetate gave a molecular ion at m/z = 535 and a fragment at 331, and malvidin 3-*O*-glucoside *p*-coumarate a molecular ion at m/z = 639 and a fragment at 331 (*Kefalas & Makris*, 2006).

As can be seen in *Table 3*, all polyphenol contents decreased significantly as a result of drying. Catechin was the most thermolabile substance exhibiting a decrease by 93.1%, whereas rutin content decreased by 79.2%. Overall, the decrease of the non-pigment polyphenols was 86.2% and of the anthocyanins 88.3%. These values are in accordance with those previously reported for RGP drying (*Goula et al.*, 2016), highlighting the detrimental effect of drying on several major polyphenolic compounds. Although the decreasing amount in non-pigment and anthocyanin compounds was almost equal, other examinations demonstrated a higher sensitivity of anthocyanins to oven drying (*Çoklar & Akbulut*, 2017; *Marchante et al.*, 2018).



Chromatograms A, B, C, and D were detected at λ= 280, 320, 365, and 520 nm respectively. Peak assignment: 1, gallic acid; 2, catechin; 3, caftaric acid; 4, rutin; 5, quercetin; 6, malvidin 3-*O*-glucoside; 7, malvidin 3-*O*-glucoside acetate; 8, malvidin 3-*O*-glucoside *p*-coumarate.

Figure 5. Representative chromatograms of the extract of RGP dried for 6 hrs at 80°C (D6/80).

Table 3. Impact of the drying process (D6/80) on major RGP polyphenols

Compound	Extraction yield (μg·g^{-1} DM)*	
	Fresh RGP	Dried RGP (D6/80)
Non-pigment polyphenols		
Gallic acid	838.36 ± 12.58	151.33 ± 2.72
Caftaric acid	842.32 ± 10.63	98.16 ± 1.77
Catechin	971.71 ± 14.58	66.69 ± 1.20
Rutin	247.20 ± 3.71	51.41 ± 0.93
Quercetin	947.71 ± 14.22	165.14 ± 2.97
Total leuco-polyphenols	3847.30	532.73
Anthocyanin pigments		
Malvidin 3-*O*-glucoside	2036.93 ± 30.55	177.98 ± 3.20
Malvidin 3-*O*-acetyl glucoside	458.41 ± 6.88	48.67 ± 0.88
Malvidin 3-*O*-*p*-coumaroyl glucoside	6643.87 ± 99.66	844.62 ± 15.20
Total anthocyanins	9139.21	1071.27

*Values reported are means (n = 3) ± standard deviation.

3.3 Effect of drying on antioxidant properties

The effect of drying on the antiradical activity (A_{AR}) was dramatic, as D6/80 brought about a decrease of 82.4% (*Figure 6A*). The effect observed for the ferric-reducing power was milder (P_R), as it was decreased by 37.2% (*Figure 6B*). Modifications in the antioxidant properties of RGP extracts as a result of drying should be normally anticipated, since they are tightly associated with the polyphenolic composition, which is largely impacted. Such a phenomenon has been dealt with by early studies, which demonstrated that increasing drying temperature resulted in the lower radical scavenging potential of RGP extracts (*Larrauri et al.*, 1997). The studies indicated herein were in concurrence, suggesting that drying may entail a significant loss of antiradical activity in RGP extracts (*Marchante et al.*, 2018), but also in P_R (*Chikwanha et al.*, 2018; *Çoklar & Akbulut*, 2017). These changes were linked with changes in the polyphenolic content. Results from investigations into other tissues, such as strawberries, were in accordance with the related observations (*Wojdyło et al.*, 2009; *Méndez-Lagunas et al.*, 2017). It should be stressed that the 82.4% decrease in A_{AR} found for the sample that received the D6/80 treatment coincided with the 86.2% decrease found for non-pigment polyphenols and the 88.3% decrease for anthocyanins. This finding might suggest

that the drop in A_{AR} actually reflected the loss of polyphenolic substances, and therefore the determination of A_{AR} could be an additional criterion in assessing drying processes.

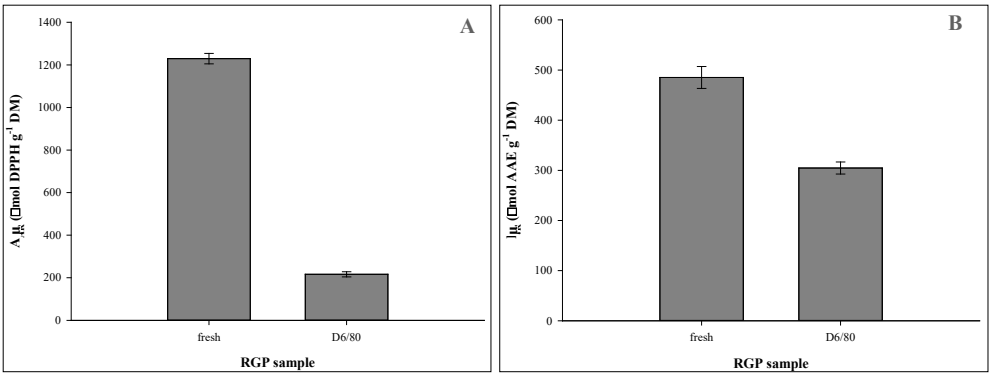


Figure 6. The effect of the drying processes (D6/80) on the antiradical activity (A) and ferric-reducing power (B) of RGP extracts

4. Conclusions

The testing of several combinations of time and temperature for RGP drying showed that D6/80 might facilitate a very effective moisture removal. However, a comparison of the sample undergone the D6/80 process with the fresh (untreated) sample revealed a drastic decline in several major polyphenols and antiradical activity. Thus, although drying is a salient compromise for long-term RGP stability, drying processes should be implemented with caution because an improper implementation of drying variables may have extremely severe effects on the polyphenolic composition and antioxidant activity. It is believed that this study could be a guide for future laboratory-scale but also large-scale studies and for the application of drying procedures with minimal impact on thermosensitive metabolites.

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Osmotic dehydration of wild garlic in sucrose–salt solution

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Abstract. Due to its nutritional and nutraceutical qualities, wild garlic (*Allium ursinum* L.) has great potential for use in the food and pharmaceutical industries. The availability of this plant is limited to the spring season, and it is perishable immediately after harvest. Osmotic dehydration (OD) is verified as an effective pre-treatment to improve sustainability by reducing the water content of raw material with minimal negative effect on its nutritive and sensorial qualities. In this study, OD of wild garlic leaves in aqueous solution of sucrose and salt was analysed at three temperatures and after diverse immersion times. The effects of the processing time and temperature on the dry matter content, water loss, and solid gain were evaluated using response surface methodology and analysis of variance. Some components in the samples were determined before and after OD. The results showed that during OD, the dry matter content of wild garlic increased from 7.91 ± 1.08% to 51.51 ± 1.34%. The maximum achieved values for water loss (0.6189 ± 0.0146 g/g i.s.) and solid gain (0.2417 ± 0.0146 g/g i.s.) indicated a good dehydration level. In the osmotically dehydrated wild garlic, the amount of analysed minerals decreased, sodium and sugar increased, and the content of protein, cellulose, and fat did not change – compared to the fresh sample.

Keywords and phrases: *Allium ursinum*, osmotic solution, mass transfer kinetics, chemical and mineral content

1. Introduction

Wild garlic (*Allium ursinum* L.), a medicinal and dietary plant with a long tradition of use, grows spontaneously on fertile soils in shady, humid places and forests of Europe and northern Asia (*Ivanova et al.*, 2009; *Krivokapić et al.*, 2020). In Serbia, during the vegetation cycle of this plant – which starts in the early spring and ends at the beginning of the summer –, this species covers a large forest complex forming a dense population, expressed in hectares (*Djurdjević et al.*, 2004; *Tomšik et al.*, 2017). The leaves are becoming increasingly popular in culinary use as vegetable, salad, spice, or an ingredient in traditional dishes (*Wu et al.*, 2009; *Šobot et al.*, 2019). Sulphur-containing compounds (predominantly alliins and methilallins) and phenolic compounds (primarily kaempferol derivatives and phenolic acids) are related to the health benefits of wild garlic and the growing interest for exploitation of this plant in the food and pharmaceutical industries (*Pejatović et al.*, 2017; *Pavlović et al.*, 2017). The limiting factor is the short period of its availability (3–3.5 months) (*Schmitt et al.*, 2005). For medical purposes, this period is limited from April to the first half of May, since the highest content of some compounds with great therapeutic potential is present in fresh leaves before flowering (*Sobolewska et al.,* 2015). Also, following harvest, this plant has a very short shelf life. *Tomšik et al.* (2016) report that a bunch of wild garlic immersed in water becomes commercially unacceptable after more than 5 days of storage at room temperature due to the very intensive yellowing and wilting and the appearance of signs of rotting of the leaves.

Drying can extend the shelf life of wild garlic and ensure its availability throughout the year, which allows for the use of this plant in food and pharmaceutical industries. It is essential to find an adequate drying method with reduced time and energy consumption, which avoids the negative effects of temperature on thermosensitive compounds and enables the preservation of sensory, nutritional, and functional properties of plants (*Filipović et al.*, 2022). Osmotic dehydration (OD) provides a decrease in drying time as well as final moisture content, the reduction of nutritional and sensory losses of the food product – at low (or at room) temperature and with low energy consumption (*Champawat et al.*, 2019; *Leahu et al.*, 2020). In this process, the immersion of raw material in a hypertonic solution leads to the flow of water through cell membranes along with natural solutes from the intercellular space of the material into the surrounding solution, by means of osmosis (*Nićetin et al.*, 2021). Simultaneously, the plant material's impregnation by the solutes from osmotic solution occurs (*Sereno et al.*, 2001; *Lončar et al.*, 2022). The two most commonly used solutes in the preparation of hypertonic solutions are sucrose and sodium chloride, but many authors proved that the combination of these two solutes is the best choice in terms of effectiveness of OD (higher water losses with lower solid gain), convenience (increase in total solution concentration

without reaching the saturation limits), and flavour (without significantly affecting osmodehydrated product taste) (*Akbarian et al.*, 2014; *Kvapil et al.*, 2020).

Due to the lack of data in literature on the OD of wild garlic in sucrose–salt solution, the aim of this work was to examine the mass transfer kinetics and chemical composition of wild garlic leaves during OD in an aqueous solution of sucrose and salt.

2. Materials and methods

The samples for investigation (*Allium ursinum*) were harvested from a forest area near Novi Sad, Serbia (45º08'34.6"N; 19º36'55.0"E), in April 2018. Fresh leaves were separated, washed under running water, wiped, and cut into squares with a dimension of 1 x 1 cm. The osmotic solution was prepared using an electric propeller mixer, which completely dissolved 350 g of commercial sodium chloride and 1,200 g of commercial sucrose in 1 kg of distilled water. These quantities were chosen based on the maximum solubility of salt and sucrose in water at room temperature in order to obtain a maximum osmotic solution concentration of 60% w/w (these three components were mixed in the following ratio: sucrose 47.04%, NaCl 13.72%, and distilled water 39.2%). Previously weighted samples (5 g) were immersed in nine laboratory vessels filled with prepared osmotic solution (100 g), maintaining in each vessel the 1:20 wild garlic/osmotic solution ratio in order to minimize changes in the solution concentration, which could lead to a local reduction in the osmotic driving force during the process. The experiments were carried out at temperatures of 20°C, 35°C, and 50°C, kept constant in an incubator (In 160, Memmert, Schwabach, Germany), under atmospheric pressure, for 1, 2.5, and 4 hours resp., with manual mixing every 15 minutes for the better homogenization of the solution and defused water from the dehydrated samples of wild garlic, thus to enhance the mass transfer. The intensity, duration, and frequency of the mixing were the same for the samples under all temperatures and immersion times in order to make the results comparable. After the selected time intervals, samples were removed from the osmotic solution and rinsed, and the free water on the surfaces was carefully collected with paper towels and measured. The content of solids in fresh and osmodehydrated samples was determined gravimetrically by drying at 105°C for 24 hrs in an oven (Instrumentaria, Sutjeska, Serbia) until a constant weight was achieved in accordance with the AOAC method No. 925.10 (2).

In order to describe the effectiveness of the mass transfer during the OD process, specific kinetic parameters (dry matter content (DMC), water loss (WL), and solid gain (SG)) were calculated using equations described by *Filipović et al.* (2017):

$$\mathrm{DMC} = \frac{m_d}{m_i} \left[100\%\right] \tag{1}$$

$$\mathrm{WL} = \frac{m_i z_i - m_f z_f}{m_i} \left[\frac{\mathrm{g}}{\mathrm{g_{i.s.}}}\right] \tag{2}$$

$$\mathrm{SG} = \frac{m_f s_f - m_i s_i}{m_i} \left[\frac{\mathrm{g}}{\mathrm{g_{i.s.}}}\right] \tag{3}$$

where m_d is the mass of dry matter for the final sample, m_i and m_f are the initial and final mass (g) of the samples respectively, z_i and z_f are the initial and final mass fraction of water (g water/g sample) respectively, s_i and s_f are the initial and final mass fraction of total solid (g total solid/g sample) respectively, and $\mathrm{g_{i.s.}}$ is the mass (g) of the initial sample. These three key parameters (DMC, WL, and SG) for the characterization of mass transfer during OD were determined for all three selected temperatures and processing times and expressed as mean values of three repeated measurements with standard deviations.

The determination of the composition of selected chemical and mineral components was conditioned by the attempt to monitor the changes caused by the mass transfer during the OD. The proximate composition of the analysed components of wild garlic sample before and after OD in the sucrose–salt solution was determined in accordance with standard AOAC methods (2000) for protein (method No. 950.36), fat (method No. 935.38), cellulose (method No. 973.18), starch content (method No. 996.11), reducing sugars (method No. 80-68), and ash (method No. 930.22). Each measurement was repeated six times. The mineral content – calcium (Ca), sodium (Na), zinc (Zn), copper (Cu), magnesium (Mg), and iron (Fe) – of samples was determined following standard methods described by AOAC (2000). Minerals were analysed by atomic absorption spectrophotometry (method No. 984.27) on a Varian Spectra AA 10 (Varian Techtron Pty Ltd., Mulgvare Victoria, Australia). Each measurement was performed in six replications.

In this study, StatSoft Statistica for Windows, ver. 10 program (Statistica, 2010) was applied for a full factorial experimental design, and the data were analysed using Response Surface Methodology (RSM) and the analysis of variance (ANOVA). The second-order polynomial (SOP) models were developed to relate the observed responses: DMC, WL, and SG with the two process variables: process time and temperature.

3. Results and discussions

In *Table 1*, mean values with standard deviations of the DMC, WL, and SG as responses of the wild garlic OD process are shown.

Table 1. Experimental results of dry matter content and kinetic parameters of wild garlic during osmotic dehydration in sucrose–salt solution

Sample	τ (h)	T (ºC)	DMC (%)	WL ($g/g_{i.s.}$)	SG ($g/g_{i.s.}$)
1.	0	20	$7.91^{a} \pm 1.08$	-	-
2.	1	20	$17.84^{b} \pm 1.37$	$0.2538^{a} \pm 0.0112$	$0.0659^{a} \pm 0.0011$
3.	2.5	20	$25.37^{c} \pm 2.03$	$0.3651^{c} \pm 0.0167$	$0.1098^{bc} \pm 0.0167$
4.	4	20	$30.36^{d\text{-}f} \pm 0.79$	$0.4086^{d} \pm 0.0058$	$0.1442^{de} \pm 0.0058$
5.	1	35	$20.80^{b} \pm 1.43$	$0.2978^{b} \pm 0.0113$	$0.0845^{ab} \pm 0.0113$
6.	2.5	35	$28.16^{cd} \pm 1.24$	$0.4100^{d} \pm 0.0088$	$0.1211^{cd} \pm 0.0088$
7.	4	35	$33.88^{f} \pm 1.19$	$0.4558^{e} \pm 0.0084$	$0.1592^{e\text{-}g} \pm 0.0084$
8.	1	50	$29.71^{de} \pm 0.09$	$0.4513^{e} \pm 0.0006$	$0.1194^{cd} \pm 0.0006$
9.	2.5	50	$41.25^{g} \pm 1.88$	$0.5783^{f} \pm 0.0110$	$0.1614^{e\text{-}h} \pm 0.0110$
10.	4	50	$51.51^{h} \pm 1.34$	$0.6189^{gh} \pm 0.0146$	$0.2417^{j} \pm 0.0146$

Notes: $^{a–h}$ – the different letters in the superscript in the same column of the table indicate statistically significant difference between values at the level of significance of $p < 0.05$ (based on post-hoc Tukey HSD test); i.s. – initial sample.

The different applied parameters of the process temperature and time resulted in a statistically significant change in all analysed responses of the OD process: DMC, WL, and SG for osmotically dehydrated wild garlic leaves, at a significance level of $p < 0.05$ (*Table 1*). The increase in the content of dry matter in samples with the progress of the OD process is the result of water diffusion from wild garlic as well as of the impregnation of sucrose and salt from solution to the dehydrated material. Higher temperatures intensify the mass transfer during OD, increasing the permeability of cell membranes and reducing the osmotic solution viscosity, which facilitates the transport of water and solutes (*Tonon et al.*, 2007). The results reveal that DMC of wild garlic increases from 7.91 ± 1.08% (fresh sample) to 51.51 ± 1.34% (sample osmodehydrated 4 hrs at 50°C). According to the results, both kinetic parameters (WL and SG) studied as an indicator of the efficiency of OD rise with the increase of process time and temperature. The maximum values for WL (0.6189 ± 0.0146 g/g i.s.) and SG (0.2417 ± 0.0146 g/g i.s.) were achieved at the end of the 4 h process at the highest temperature of 50°C and indicated a good dehydration level. Similar results were also reported for the OD of celery leaves in aqueous solution of sucrose and salt – WL of 0.712 ± 0.006 g/g i.s. and SG of

0.240 ± 0.002 g/g i.s. (*Nićetin*, 2017) – and for nettle leaves osmodehydrated in the same solution – WL of 0.487 ± 0.001 g/g i.s. and SG of 0.260 ± 0.002 g/g i.s. (*Knežević et al.*, 2015). OD performed in the same solution but for animal material (pork meat) resulted in a similar value for SG (0.289 ± 0.012 g/g i.s), as observed by *Ćurčić et al.* (2014).

Table 2. ANOVA table of response values of the osmotic dehydration of wild garlic in sucrose–salt solution

Technological parameters	Term	df*	Sum of squares		
			DMC	WL	SG
Time	Linear	1	1483.085*	0.362417*	0.050002*
	Quadratic	1	89.544*	0.082539*	0.002231
Temperature	Linear	1	326.999*	0.051414*	0.005639*
	Quadratic	1	38.408	0.005052	0.000529
Cross product	Time x Temp.	1	107.724*	0.009007	0.002020
Error	Residual variance	6	52.311	0.023633	0.002262
	Total sum of squares	11	2051.315	0.521838	0.061641
	R^2		0.9745	0.95471	0.9633

* Statistically significant at a level of significance of $p < 0.05$; * df – degrees of freedom.

Table 2 shows the results of ANOVA of the RSM models that were developed on the basis of the experimental results shown in *Table 1*. Based on these results, the statistically significant effects of process parameters (time, temperature) as well as their interdependence on the responses of the mathematical model (dry matter content, water loss, solid gain) were analysed. The ANOVA test indicated that the values of the DMC, WL, and SG were statistically significantly ($p < 0.05$) influenced by both process parameters (linear terms of time and temperature), time being the most influential parameter. The quadratic term for time contributed statistically significantly to the formation of the SOP model for the prediction of DMC and WL, as a consequence of reducing mass transfer rates with the flow time of the process. The cross product of time and temperature was statistically significant ($p < 0.05$) and contributed to the forming of the SOP model only for DMC. Residual variances were not statistically significant, demonstrating that the applied model for DMC, WL, and SG was adequate for the OD of wild garlic leaves, with a high level of determination coefficient R^2 (0.97454, 0.95471, 0.9633). This indicated a good fitting of the SOP model with the obtained experimental values.

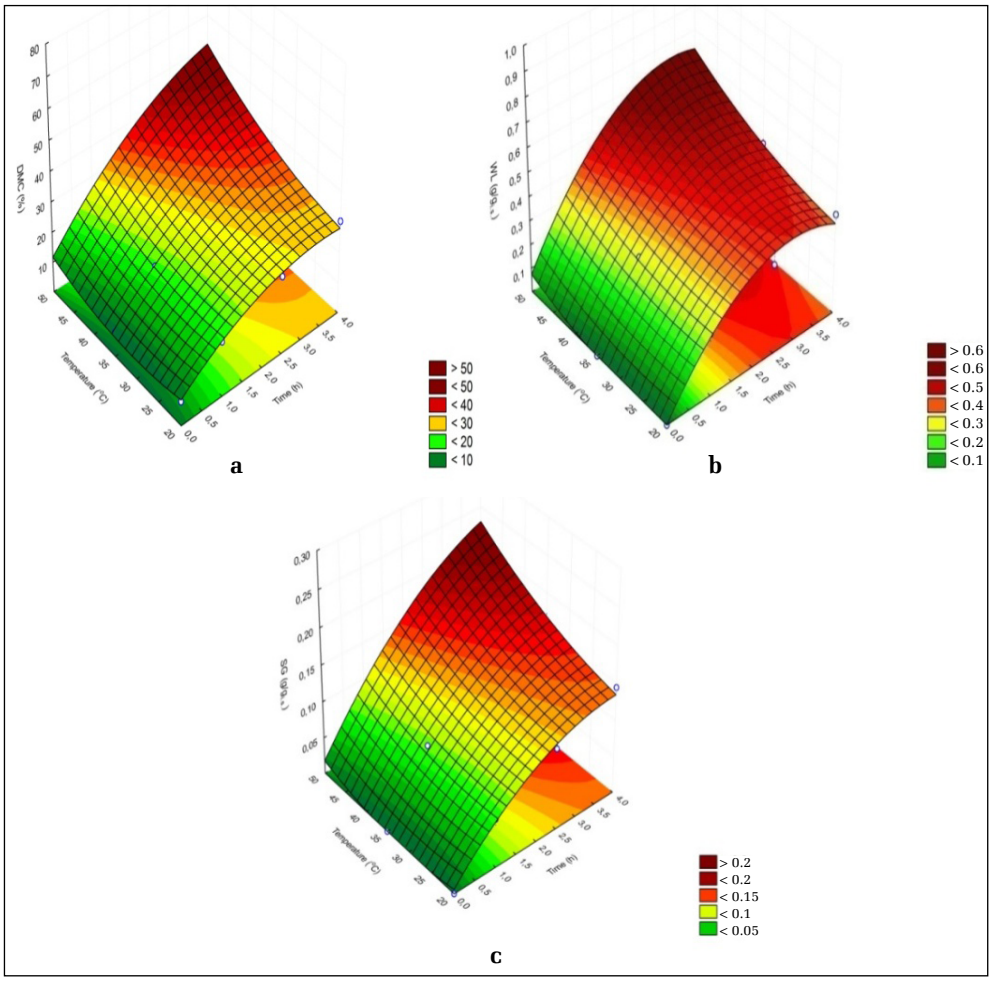

Figure 1. Change in dry matter content (a), water loss (b), and solid gain (c) depending on temperature and time during the osmotic dehydration of wild garlic

Figures 1 (a, b, c) provide graphical representations of developed mathematical models for all responses of the process of OD of wild garlic in aqueous osmotic solution of sucrose and salt, from which the dependence of changes in the observed response on changes in time and temperature can be monitored. It can be seen from the figures that the increase in the value of both process parameters (time and temperature) led to an increase in the values of DMC, WL, and SG. The graphs can also quantify the greater influence of time change as compared to temperature change on the change of all responses during the OD process of wild garlic, which is in accordance with the results of the ANOVA test (*Table 2*).

The uptake of dissolved substances from osmotic solution and the leaching of food constituents (such as vitamins and minerals) result in the alternation of the composition of the original product, which could adversely influence the nutritional profile and organoleptic attributes (*Akbarian et al.*, 2014). High values of SG in wild garlic are undesirable since the increased uptake of sucrose is associated with the occurrence of diabetes and dental problems and that of salt with hypertension (*Kaur et al.*, 2022). In order to achieve the greatest possible water loss (0.409 ± 0.006 g/g i.s) with the lowest sugar and salt impregnation (0.144 ± 0.006 g/g i.s), the sample dehydrated at 20°C after 4 hours was selected for examining the influence of OD in sucrose–salt solution on the chemical composition of wild garlic.

The amounts of chemical compounds were expressed as the percentage of dry matter of wild garlic. Of the total 7.91% of dry matter in fresh wild garlic, it was determined that proteins participate with 33.97%, cellulose with 32.12%, lipids with 0.11%, and ash with 9.80%. Similar to the presented results, *Piatkowska et al.* (2015) reported that the content of dry matter in wild garlic was 7.9%, of which 17.72% protein content, 32.9% fibre, 7% fat, and 11.26% minerals as ash. In the case of osmotically dehydrated samples, the total percentage of dry matter is reduced by 14.4% of the dry matter (SG) that was adopted from the osmotic solution. Based on the results in *Table 3*, it is obvious that the content of protein, cellulose, and fat in comparison with the dry matter in the fresh samples remained unchanged. On the other hand, as a consequence of sugar uptake through mass transfer during OD, osmodehydrated wild garlic contained 10% of total sugars. Also, the ash content in the osmotically dehydrated sample increased by about 4% as a result of salt (sodium) intake.

In the investigation by *Vučić et al.* (2018), the content of the examined macroelements in the samples of wild garlic from different locations was in the range of 317–335 mg/kg for magnesium (Mg), 31–33 mg/kg for sodium (Na), and 1,532–1,559 mg/kg for calcium (Ca), and the content of the examined microelement was in the range of 13.9–15.6 mg/kg for iron (Fe), 2.3–2.6 for zinc (Zn), and 1.6–1.9 mg/kg for copper (Cu). This is in accordance with the presented results, but the determined amounts of Mg, Zn, and Cu were higher in this study. When the composition of mineral components was expressed in the same way as of the chemical components (percentages reduced by 14.4% given by the dry matter content of fresh wild garlic), the amounts of Mg, Zn, Cu, and Fe were reduced by about 30% and that of Ca by about 40%. This finding is in accordance with that reported by *Cvetković et al.* (2019), who find the content decreased of Fe, Cu, Mg, and Ca at 30–60% after the osmotic dehydration of cabbage in sucrose–salt solution. The reduction of mineral components is a result of the diffusion of part of the cellular juices from the wild garlic tissue into the osmotic solution. On the other hand, an increase in sodium and total sugars reflects the penetration of the solutes from the osmotic solution during the OD.

Table 3. Changes in chemical and mineral components in wild garlic after osmotic dehydration in sucrose–salt solution

Chemical component	Fresh wild garlic	Osmodehydrated wild garlic (% d. m.)	Mineral matter	Fresh wild garlic	Osmodehydrated wild garlic (mg/kg)
Protein	33.97 ± 1.27[a]	29.01 ± 0.74[b]	Zn	5.31 ± 0.37[a]	2.81 ± 0.11[b]
Starch	0.00 ± 0.00[a]	0.00 ± 0.00[a]	Cu	1.63 ± 0.10[a]	0.86 ± 0.08[b]
Total sugars	0.00 ± 0.00[a]	10.08 ± 0.26[b]	Mg	911.77 ± 89.19[a]	501.77 ± 70.44[b]
Cellulose	32.12 ± 1.81[a]	27.03 ± 0.41[b]	Na	32.87 ± 2.01[a]	4356 ± 1.20[b]
Fat	0.11 ± 0.01[a]	0.09 ± 0.01[b]	Ca	1432.01 ± 93.91[a]	770.46 ± 94.58[b]
Ash	9.80 ± 0.51[a]	12.95 ± 1.83[b]	Fe	18.71 ± 2.01[a]	10.03 ± 0.99[a]

Note: [a–b] – the different letters in the superscript in the same column of the table indicate statistically significant difference between values at the level of significance of $p < 0.05$ (based on post-hoc Tukey HSD test).

Having an extended shelf life, the obtained partially dehydrated wild garlic leaves could be used for direct consumption or in combination with various food products. Osmotically dehydrated wild garlic in a solution of sucrose and salt is suitable as an ingredient in food formulations such as yogurt, sauces, bakery products, and snacks for the nutritional and sensory improvement of these products. *Šobot et al.* (2019) proposed a new product, a biscuit with osmotically dehydrated wild garlic in molasses. Unlike osmodehydrated wild garlic, adding fresh leaves can adversely affect the texture of food products. Also, the increased content of dry matter in osmodehydrated wild garlic allows for greater quantities to be added.

4. Conclusions

Based on the above-discussed, it can be concluded that:

– An increase in processing temperature and duration of osmotic dehydration resulted in improved mass transfer during the process. Accordingly, the maximum values obtained for dry matter content, water loss, and solid gain were achieved at the end of 4 hours of the process at the highest temperature (50°C), indicating a good level of dehydration.

– Statistical analysis confirmed that the values of investigated kinetic parameters were influenced by both process parameters (time and temperature), with time being the most influential parameter.

– The content of mineral components in osmodehydrated wild garlic decreased, whereas sodium and sucrose content increased as a consequence of mass transfer during the osmotic dehydration in the sucrose–salt solution.

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Milk powder incorporation in the cereal-based Nepalese indigenous food *bhakka* and its quality assessment

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Abstract. This work aims to study the effect of milk powder incorporation on the nutritional and sensory quality of our traditional indigenous food, *bhakka*. Different samples were prepared replacing the rice flour by milk powder at different ratios (2.5:97.5, 5:95, 7.5:92.5, 10:90). *Bhakka* prepared from 100% rice flour was considered as control. Sensory-related results showed that 7.5% milk powder and 92.5% rice flour can be mixed to prepare an acceptable quality. The proximate analysis showed protein content of 5.18% in rice flour *bhakka* and 6.7% in milk-powder-incorporated *bhakka*. The milk-powder-incorporated *bhakka* had a fat content of 1.51%, crude fibre content of 0.18%, crude protein content of 6.70%, total ash content of 0.44%, and carbohydrate content of 62.3%, which was higher than that of rice flour *bhakka*. The product prepared can help in combating protein–energy malnutrition, adds a new taste as well as value to our traditional indigenous food.

Keywords and phrases: Bhakka, rice flour, milk powder, proximate composition, indigenous food, sensory evaluation

1. Introduction

Traditional foods are foods and dishes passed down through generations (Kristbergsson & Oliveira, 2016) or consumed over many generations (Saunders, 2010). Traditional foods and dishes are traditional in nature and may have a historical precedent in a national, regional, or local cuisine (Kristbergsson & Oliveira, 2016).

Bhakka is a steam-cooked cereal product, prepared from coarse rice flour; it is indigenous to the *Tharu* community of the Terai but is enjoyed by all (Dangal et al., 2021). *Bhakka*, a simple fluffy rice flour steamed cake is the indigenous and traditional delicacy as well as seasonal income source for people of the *Tharu* community, including the *Rajbanshi* and *Dhimal* communities living on the eastern plains of Nepal, especially in Morang District. Nowadays, it is becoming increasingly popular in other regions and cultures too. No specific legend of naming '*Bhakka*' has been identified yet, but they say it was named so because it is a food cooked with steam. Vapour is 'Bhaff' in the *Tharu* language and eating is 'Khabbe'. So, it is said that 'Bhakha' later became '*Bhakka*' (Rai, 2004). *Bhakka* is generally prepared from Kanchhi rice flour, and selling it is a seasonal business that generally starts in October and ends in February. It is prepared traditionally by soaking cleaned whole rice in water and hand-pounded (in *okhli* or *dhiki*) to fine flour mass to obtain rice flour after the complete draining of water. Nowadays, soaked rice is milled to obtain fine rice flour. The flour obtained is used to make dough of desired property, press-sieved through the wire screens of small mesh sizes or through the mosquito curtain to obtain small granules which are then loosely filled into moulds (small plate, bowl, bicycle bell), and atmospheric steam cooking is done for about 2-3 min after wrapping the shaped mass by muslin cloth (Rai, 2004).

Bhakka is Nepal's traditional indigenous food, which is cheap and affordable by nearly every group of people. Therefore, such foods should be as rich as possible in nutritional terms. Rice flour used to make *bhakka* contains insufficient quantities of vitamin A, iron, and lysine, an essential amino acid (Sautter et al., 2006). The incorporation of milk powder increases the protein content of *bhakka* and fortifies it with lysine as well as enriches it with all kinds of essential amino acids. Milk powder is also richer in different minerals and vitamins compared to rice.

This study was therefore necessary in order to address the deficiencies in *bhakka* rice flour by the partial incorporation of milk powder. The present study aims to investigate the effects of the incorporation of milk powder in the Nepalese indigenous food *bhakka* and helps to introduce *bhakka* to the people as an important traditional and indigenous food of ours and to make them know its nutritional content.

2. Materials and methods

Material procurement

New variety rice (*Oryzae sativa* var. *Ranjeet*) and milk powder (Nestlé EveryDay) were collected from the local market of Dharan, Sunsari.

Methods

Preparation of rice flour

The required amount of the new rice variety (*Oryzae sativa* var. *Ranjeet*) was weighted and cleaned in running water. The cleaned rice was soaked at 20°C for 10 min, as shown in *Fig. 1*. Then, the water was drained off into a container with holes at its base. After draining the water, the rice was grinded in an electric grinder for milling purposes, and the obtained rice flour was sifted in a sieve with a mesh size of d = 1 mm to separate the broken rice, and so fine flour was obtained.

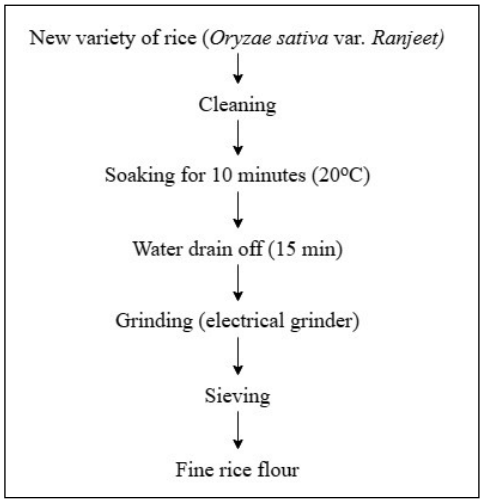

Figure 1. Flowchart for the preparation of rice flour

Preparation of rice flour–milk powder mixes

Four blends were prepared by mixing milk powder with rice flour using an electric blender in the percentage ratios of 2.5:97.5, 5:95, 7.5:92.5, and 10:90, while 100% rice flour served as the control (*Table 1*).

Table 1. Formulation of flour blend

Samples	Rice flour (%)	Milk powder (%)
R	100	0
A	97.5	2.5
B	95	5
C	92.5	7.5
D	90	10

Preparation of bhakka

The method described by Rai (2004) was used with slight modifications in the production of *bhakka*, as shown in *Fig. 2*. The ingredients (flour mix and water) were thoroughly mixed in a bowl by hand to obtain a dough with 29% moisture content. The dough was then forced through the wire net to obtain the granular form of the mixture. Then it was shaped in a bowl, and the excess was removed by hand or by a knife, without pressing. The bowl was then wrapped in a muslin cloth and steamed in inverted position for 2-3 min under atmospheric pressure. The prepared *bhakka* was taken for further analysis.

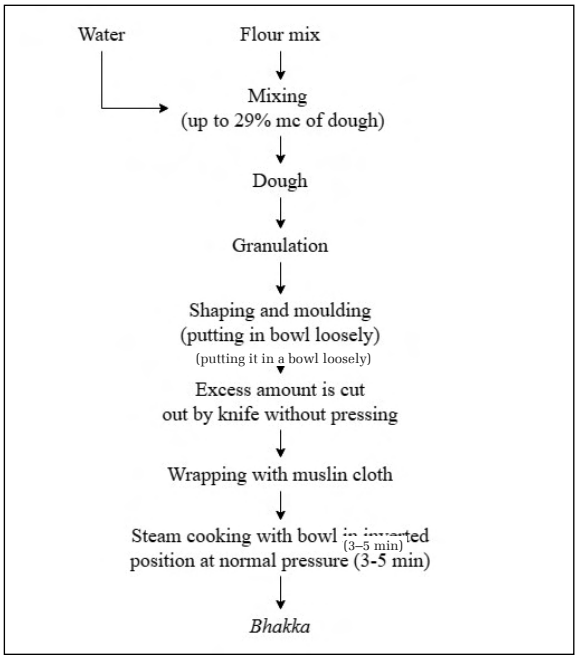

Figure 2. Flowsheet for the preparation of *bhakka*

Determination of proximate composition

Measurements of moisture content, crude fat, crude protein, crude fibre, and carbohydrate content of the rice flour, milk powder, and the prepared *bhakka* samples were carried out using Ranganna's method (1986), and ash content was determined by using the method described in AOAC (2012).

Physical analysis of the bhakkas produced

The diameter was measured by placing three *bhakkas* edge to edge with the help of a scale, rotating them by 90 degrees, measuring again the diameter of the three *bhakkas,* and then taking the average value. Thickness was measured by stacking three *bhakkas* on top of each other and taking the average thickness (Baljeet et al., 2010). The weight of the biscuits was measured as an average of three individual *bhakka*s with the aid of a digital balance (Ayo et al., 2007). The yield of *bhakka* was determined with the method described by Lynch (2007):

$$\text{Yield } \% = \frac{\text{Product weight}}{\text{Raw material weight (Except added water)}} \times 100 \quad (1)$$

Sensory evaluation

Sensory evaluation was determined by a ten-member committee consisting of the teaching staff and students of the Central Department of Food Technology, Dharan, Nepal. Committee members were either regular or occasional consumers of *bhakka*. The samples prepared from each flour blend were presented in coded, disposable plates. Bottled water was provided to the panelists to rinse their mouth. They were instructed to evaluate the coded samples for flavour, appearance, texture, and overall acceptability. Each sensory attribute was rated on a 9-point Hedonic scale (1 = disliked extremely, 5 = neither like nor dislike, while 9 = liked extremely) (*Ranganna*, 2005).

Statistical analysis

All data obtained from the sensory analysis were subjected to the Kruskal–Wallis H test using the statistical software R 64 4.0.2, which was developed by R core team, and then were presented as mean values with standard deviations. The statistical analysis was run with a confidence level of 95%. Differences between treatments were established with Dunn's Multiple-Comparison test, and the best sample was subjected to proximate analysis and physical analysis.

3. Results and discussion

The proximate composition of raw materials

The proximate compositions of rice flour and milk powder were analysed and are presented in *Table 2*.

Table 2. Proximate composition of rice flour and milk powder

Parameter	Rice flour (%)	Milk powder (%)
Moisture	12.10 ± 0.417	2.26 ± 0.110
Crude protein	7.18 ± 0.206	20.32 ± 0.960
Crude fat	0.20 ± 0.014	15.74 ± 0.790
Crude fibre	0.25 ± 0.018	0 ± 0.0
Total ash	0.40 ± 0.013	5.10 ± 0.205
Carbohydrate	79.87 ± 1.120	56.58 ± 1.210

Note: The values are the means of triplicate samples, and the values after "±" are standard deviations.

The crude protein, moisture, crude fat, crude fibre, and total ash content of the rice flour resulted from the analysis are shown in *Table 2*. The values fall in the range of flour as described by *Arora* (1980). Also, milk powder was analysed for its proximate composition. The obtained values can be seen in *Table 2*. The protein content of rice flour was found to be 7.17%, while it was observed to be 20.32% in milk powder. Also, the fat content of both constituents was observed: for rice flour, it was found to be 0.19% and 15.74% for milk powder.

Sensory analysis

All the scores from sensory analysis were summed up, and the best varieties were established by statistical analysis. The Kruskal–Wallis H test followed by Dunn's test at 95% level of confidence ($p < 0.05$) showed that the control and samples A, B, C, and D were significantly different from each other. The mean sensory evaluation scores of the *bhakka* samples are shown in *Table 3*.

The Kruskal–Wallis H test (at $p \leq 0.05$) followed by Dunn's Multiple-Comparison Test was carried out to find out the significant differences between the control and four formulations. The results obtained are shown in *Fig. 3*.

Table 3. Mean sensory scores of different samples of milk-powder-incorporated *bhakka*

	Parameters			
Formulation	**Colour**	**Texture**	**Flavour**	**Overall acceptability**
R	$6.3^{bc} \pm 1.06$	$5.7^{a} \pm 0.67$	$5.7^{d} \pm 0.67$	$6.1^{ac} \pm 0.57$
A	$6.1^{b} \pm 0.74$	$6.0^{a} \pm 0.67$	$6.2^{ad} \pm 0.79$	$5.9^{a} \pm 0.74$
B	$6.8^{ac} \pm 0.63$	$6.4^{a} \pm 0.52$	$7.0^{ac} \pm 0.82$	$6.7^{abc} \pm 0.48$
C	$7.6^{a} \pm 0.52$	$7.7^{b} \pm 0.48$	$7.5^{bc} \pm 0.71$	$7.9^{b} \pm 0.57$
D	$6.5^{bc} \pm 0.53$	$6.4^{a} \pm 0.52$	$7.6^{c} \pm 0.84$	$6.9^{ab} \pm 0.74$

Note: Mean ± Standard deviation of sensory evaluation score. Means with the same superscripts within a column are not significantly different ($p < 0.05$).
R = Control sample (100% rice flour *bhakka*);
A = Milk-powder-incorporated *bhakka* (2.5:97.5);
B = Milk-powder-incorporated *bhakka* (5:95);
C = Milk-powder-incorporated *bhakka* (7.5:92.5);
D = Milk-powder-incorporated *bhakka* (10:90).

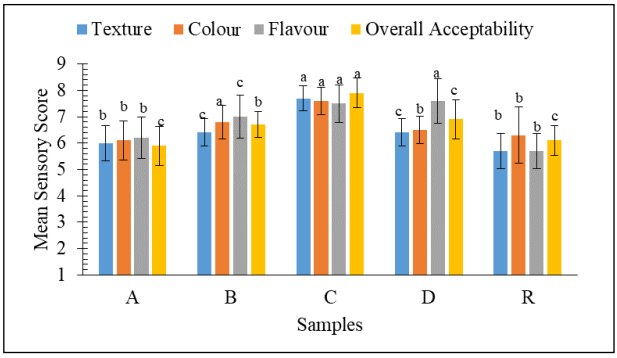

Figure 3. Mean sensory scores of different samples of *bhakka*

Colour

The colour of R showed no significant difference between A, B, and D. The sensory colour scores showed greater acceptability for sample C, which had an adequate amount of milk powder, which could be due to an increase in fat content, as the content of fat in *milk powder recipes* will also affect the colour of milk powder, and, generally, the colour of whole milk powder is yellow (Samantha, 2019).

Texture

The texture of R, A, B, and D showed no significant differences between one another, while C was significantly different from R, A, B, and D. The scores from the sensory analysis showed sample C as the best. This might be due to balancing the decreasing proportion of amylose by increasing the proportion of protein. Amylose was found to positively correlate with rice texture (*Juliano*, 1965; *Williams et al.*, 1958). High protein content in rice causes a reduction in stickiness after cooking (Primo et al., 1962).

Flavour

There was no significant difference between the flavour of A and R and that of A and B. Samples B, C, and D also showed no significant difference between one another. However, the mean sensory score shows sample D as superior among four samples. This might be due to the higher proportion of the added milk powder.

Overall acceptability

The overall acceptability of R showed no significant difference between A, B, and D. The overall acceptability mean showed C as the superior. Samples A, B, and the control R were found to be stickier, whereas sample D was found to have more firm texture. This might be due to the increase in protein content, as high protein content in rice causes a reduction in stickiness after cooking (Primo et al., 1962).

Proximate analysis of milk-powder-incorporated bhakka *and rice flour* bhakka

The proximate analysis of the best sample, C, as suggested by the sensory analysis, and of normal rice flour *bhakka* was carried out. The result is tabulated in *Table 4*.

The ash content of *bhakka* was increased in milk-powder-incorporated *bhakka*. The increase in ash content may be due to the high mineral content of milk powder. The moisture ranged from 28.82% in milk-powder-incorporated *bhakka* to 36.63% in rice *bhakka*. It is because 36% moisture content was to be maintained to prepare self-standing dough for rice *bhakka*, whereas for preparing self-standing dough of milk-powder-incorporated *bhakka*, only 29% moisture was to be maintained. The fat content of milk-powder-incorporated *bhakka* was higher than that of rice *bhakka*. No definite trend in increase or decrease in crude fibre content was observed. *Bhakka* showed an increase in protein content when milk powder concentration was increased.

Table 4. Proximate analysis of milk-powder-incorporated and normal rice *bhakka*

Parameters	Milk-powder-incorporated *bhakka*	Rice *bhakka*
Moisture (%)	28.82 ± 0.22	36.63 ± 0.470
Crude fat (%)	1.51 ± 0.010	0.14 ± 0.010
Crude protein (%)	6.70 ± 0.112	5.18 ± 0.020
Crude fibre (%)	0.18 ± 0.002	0.18 ± 0.003
Total ash (%)	0.45 ± 0.014	0.28 ± 0.002
Carbohydrate (%)	62.34 ± 0.344	57.59 ± 0.490
Total energy (kJ/100g)	1,212.31 ± 0.420	1,055.74 ± 0.475

Note: The values are the means of triplicate samples, and the values after "±" are standard deviations.

Hence, the incorporation of milk powder improved the nutritional composition of *bhakka* in terms of protein and fat, which may contribute towards combating protein–energy malnutrition. It also adds a new taste and value to our indigenous food *bhakka*.

Physical analysis

Bhakka was analysed for its physical properties, diameter, weight, and thickness, which were found to be d = 9.27 cm, m = 28.73 g, and t = 1.57cm respectively.

The yield of bhakka

The yield of *bhakka* was found to be 124.53%. The yield of the final product can be increased by minimizing processing loss and by taking the production to mass scale.

4. Conclusions

The results obtained from the sensory analysis of the flour mix showed that the composite flour blends could be suitable for *bhakka* production. This study also revealed that the proximate composition of *bhakka* produced from the flour blends is better compared to that of made solely from rice flour, and hence milk powder can be incorporated up to 7.5% substitution level based on the sensory score. The energy value was found to be 1,212.3 kJ/100 g of milk-powder-incorporated *bhakka.* The product prepared could help in combating protein–energy malnutrition, adds a new taste as well as adds value to our traditional indigenous food.

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The role of circular economy in food waste management in fulfilling the United Nations' sustainable development goals

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Abstract. Based on the UNEP Food Waste Index Report 2021, approximately 931 million tons of food waste were generated in 2019, with nearly 570 million tons of that waste occurring in households. Hunger affects more than 800 million people worldwide. Furthermore, the non-consumption of food accounts for 8-10% of global greenhouse gas emissions. Therefore, food waste generation has significant environmental, societal, and economic consequences. The circular economy (CE) is an economic model that seeks to reduce waste and extend resource life. The purpose of this study is to examine and present the connections between the CE, the Sustainable Development Goals (SDGs), and food waste management. Food waste management is commonly regarded as an environmental issue. Still, it also offers vital economic and social benefits for sustainable development. The first section of the research looks at the function of food waste management in the transition to a circular economy. The second part of this study examines the SDGs in food waste management and circular economy. The findings revealed that the most significant connections and synergies between food waste management, circular economy practices, and SDG targets could be found in SDG 2 (Zero Hunger) and SDG 12 (Responsible Consumption and Production). Both goals have high scores for direct and indirect contributions.

Keywords and phrases: economic development, responsible consumption and production, global goals, zero hungerwaste hierarchy

1. Introduction

More than 800 million people worldwide suffer from hunger, and the number of hungry people has risen since 2014 (*Kruchten & Eijk*, 2020). Moreover, global food crises will be compounded by the unfolding effects of the COVID-19 pandemic (*Kansiime et al.*, 2021) and with the projection that the global population will reach 8.5 billion people in 2030 (*UN*, 2019).

Food waste is considered a global issue, with roughly one third of all food produced for human consumption being lost or wasted (*Reynolds et al.*, 2020). On a global scale, around 931 million tons of food were wasted in 2019, with nearly 570 million tons of waste occurring at the household level (*UNEP*, 2021). Approximately 88 million tons of food waste are generated annually in the EU, with related costs valued at 143 million euros (*Stenmarck et al.*, 2016). Food waste is responsible for resource losses, greenhouse gas (GHG) emissions, and economic loss (*Niu et al.*, 2022). Apart from the fact that food wastage causes severe financial losses, both for personal consumption and the national economy, it also causes loss of life-supporting nutrition and consumes scarce resources (land, water, and energy) expended for the production, processing, and distribution of food (*Rainer Bräutigam et al.*, 2014). Furthermore, global food loss and waste are responsible for 8-10% of anthropogenic greenhouse gas emissions (*Costa et al.*, 2022).

Circular economy (CE) presents an evolving umbrella concept for closing material loops to improve environmental performance (*Castro et al.*, 2022). The CE concept replaces the linear economy concept to fulfil the need for an alternative approach, which values raw materials differently. Transition to circular economy contributes to utilizing resources' value to the maximum and their retention in use for as long as possible. Materials and products in a CE are meant to reduce waste and are reused, recycled, or recovered (*ISWA*, 2015). As a result, CE is gaining traction worldwide to achieve local, national, and global sustainability goals. Nonetheless, developed countries receive the most of the attention, with developing countries receiving only a tiny portion (*Azizuddin et al.*, 2021).

Transition to a circular economy can be achievable and sustainable if it supports the increased demands of a current growing population while addressing the environmental and resource problems that future generations will face (*Monkelbaan*, 2021). In a circular food economy, waste management aims to produce resources: biofertilizers and biofuels. Biogas is the most valuable product, a non-fossil fuel used for commercial and private vehicles and heating (*Holmberg & Ideland*, 2021).

The 2030 Agenda for Sustainable Development was adopted by the UN General Assembly on 25 Sept 2015. It includes 17 Sustainable Development Goals (SDGs), which set quantitative goals in the social, economic, and environmental dimensions of sustainable development (*UN*, 2015). By 2030, SDGs should be achieved. Even though there are no specific SDGs on waste, several goals and related targets include

direct, moderately direct, and indirect references to waste (*Prokić et al.*, 2016). On the other hand, SDG 2 (Zero Hunger) and SDG 12 (Sustainable Consumption and Production) set targets to deal with food waste with several other SDGs and related targets that are moderately directly and indirectly related to food waste.

This paper provides a framework that establishes detailed interrelationships between food waste management, the CE, and SDGs to direct future sustainable development research, policies, and innovations. Food waste management is generally treated as an environmental challenge, but it also has significant benefits for the economic and social goals of sustainable development.

2. Materials and methods

This study used a desk review methodology to address research objectives (*Bowron & Weber*, 2019). The analysis of scientific literature is developed using the Scopus database of academic publications (*Snyder*, 2019). The research was conducted in two phases. The first phase presents a scientific literature review to examine the link between the CE and food waste. The review highlights how food waste management can contribute to the CE transition and how implementing the CE concept reduces food waste. The second phase of this research analyses the SDGs and related targets in the context of CE and food waste management.

3. Results and discussion

3.1. Contribution of food waste management to the circular economy

Sustainable food waste management is a global issue with a high priority for enhancing food security and conserving natural resources and ecosystems (*Zan et al.*, 2022). Food waste is expected in the early stages of the supply chain in developing and underdeveloped countries where poverty is high. However, waste generation is the largest in the later supply chain stages due to supermarket practices or consumer waste in developed countries (*Joubert & Jokonya*, 2021). On a worldwide scale, food supply chains place a tremendous burden on freshwater resources while also returning excess nitrogen and phosphorus, endangering the health of sensitive ecosystems (*Reynolds et al.*, 2020).

Moreover, food waste management causes more ecological damage, mainly when landfilled, as is a common practice in many countries. In addition, food waste decomposes, creating methane, a potent greenhouse gas in the anaerobic landfill (*Babbitt*, 2017). Therefore, the EU launched European Directive (EU) 2018/851 to avoid waste treatment that locks in resources at the lower levels of the waste

hierarchy. The directive aims to increase preparation for reuse and recycling rates, enable high-quality recycling, and raise the uptake of quality secondary raw materials. The directive is the New Waste Framework Directive (WFD), included in the Circular Economy Package (*EU*, 2018). As of 1 January 2024, the WFD requires that bio-waste, including food waste, be collected separately. Belgium, for instance, has already achieved the 2020 target of 50% recycling rates and has eliminated landfilling biodegradable waste (*Favoino & Giavini*, 2020).

In 2014, the EU adopted a document, *Towards a Circular Economy: A Zero Waste Programme for Europe*, to encourage the transition of the European economy from the linear to the circular model. This document treats waste as a resource, contributing to the circular economy's key concept of "closing the loop". In addition, the EU established an Action Plan for the Circular Economy in 2015, which sets a clear and ambitious EU mission to assist in the transition to a circular economy. Circular economy is regarded as a strategy to safeguard businesses from resource scarcity and price volatility while increasing the EU's competitiveness, creating new economic opportunities, and encouraging more inventive and efficient production processes. The Action Plan is oriented towards the EU. However, it confirms that all stakeholders (Member States, regions, cities, businesses, and individuals) must be engaged in successfully implementing the circular economy. The Action Plan also promotes the Agenda 21 goals, notably SDG 12, which encourages sustainable production and consumption (*UNDP*, 2020).

Conventional waste management practices dispose of or incinerate food waste, contributing to the degradation of the environment while misusing bio-waste, a valuable resource with a great potential for reuse. As a result, decreasing food waste can benefit the environment and society. Furthermore, new business models can arise by using a circular economy strategy to reduce, reuse, and recycle food waste and gain financial benefits from what would otherwise be discarded (*KPMG*, 2020).

CE mainly appears in the literature through three main actions: the 3Rs rule (*Zan et al.*, 2022). Food waste management 3Rs can be presented as follows: Reduce – minimize the amount of material that is thrown away, Reuse – redistribution of excess food, and Recycle – creating new value from inedible by-products and food waste. As shown in *Fig. 1*, the food waste hierarchy is highly associated with CE-based rules. Based on environmental sustainability, a waste hierarchy prioritizes waste treatment actions from the most preferred to the least preferred. The EU WFD can be applied to food waste but should be slightly modified to take account of the particularities of food (*Lombardi & Costantino*, 2021; *EU*, 2018).

In the context of food waste, the first element of CE is food waste prevention (*Zan et al.*, 2022). Preventing food waste generation is possible by using all activities that reduce the amount of waste. It can be achieved by preventing food waste generation in primary production, processing and production, retail and other forms of food distribution, restaurants and food services, and households.

Preventing food waste is the most effective method of waste management; although prevention is a technique that involves the most significant effort when established, it achieves indisputable and best results in terms of sustainability. The second element of CE refers to the more efficient use of generated waste by carrying out various reuse or recycling activities, i.e. converting waste into resources and not ending up in a landfill. Several ways of processing this waste, ranging from the most environmentally and socially desirable, such as donating food to banks or the Red Cross, to processing into animal feed, electricity, and composting.

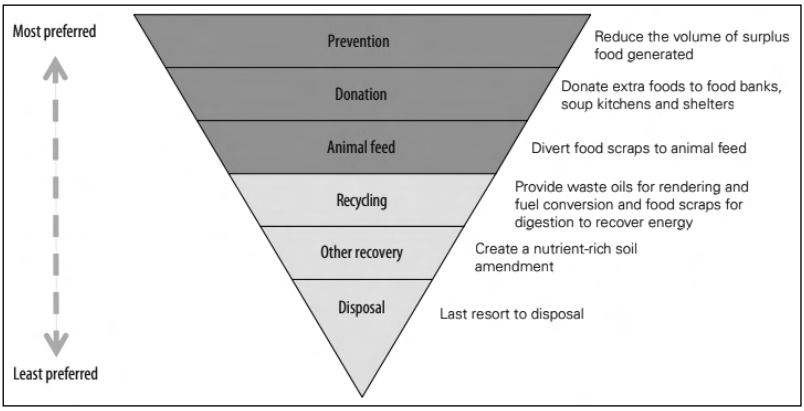


Figure 1. Food waste hierarchy (*EU*, 2018; *KPMG*, 2020)

Throwing food away is the most undesirable solution because it represents a pure economic loss, with severe degradation of the environment. Therefore, waste separation is a necessary condition in achieving a circular economy. The installation and use of appropriate containers to separate this type of waste at its origin is the initial step that must be done to create the conditions for achieving a circular economy (*Favoino & Giavini*, 2020).

Despite environmental policy being a global economic policy, only 8.6% of the world is circular currently, which is a negative trend. A higher degree of circularity can be achieved through transferring global trends to national, regional, and commercial levels. Also, it can be accomplished by establishing implementation models and measures to monitor the transition (such as penalty policies). Additionally, it can be reached by providing accessible education and know-how and involving various stakeholders in forming a global coalition for circular economy (*UNDP*, 2020). Expanded imports of recyclable waste to developing countries can also create new possibilities. For instance, such imports enhance demand for emerging sectors such as repair and recycling, improving domestic waste management. It can also generate a significant number of jobs, as product repair tends to be more labour-intensive than manufacturing from raw materials (*Monkelbaan*, 2021). Furthermore, innovative

business models that contain digital technology can assist in managing food waste at the household and retail levels, which amounts to roughly 88 million tonnes per year in the EU, or 173 kg/person/year (*Stenmarck et al.*, 2016). Globally, food loss and waste are responsible for about 8% of GHG emissions, and all countries have to minimize this figure (*PACE*, 2021).

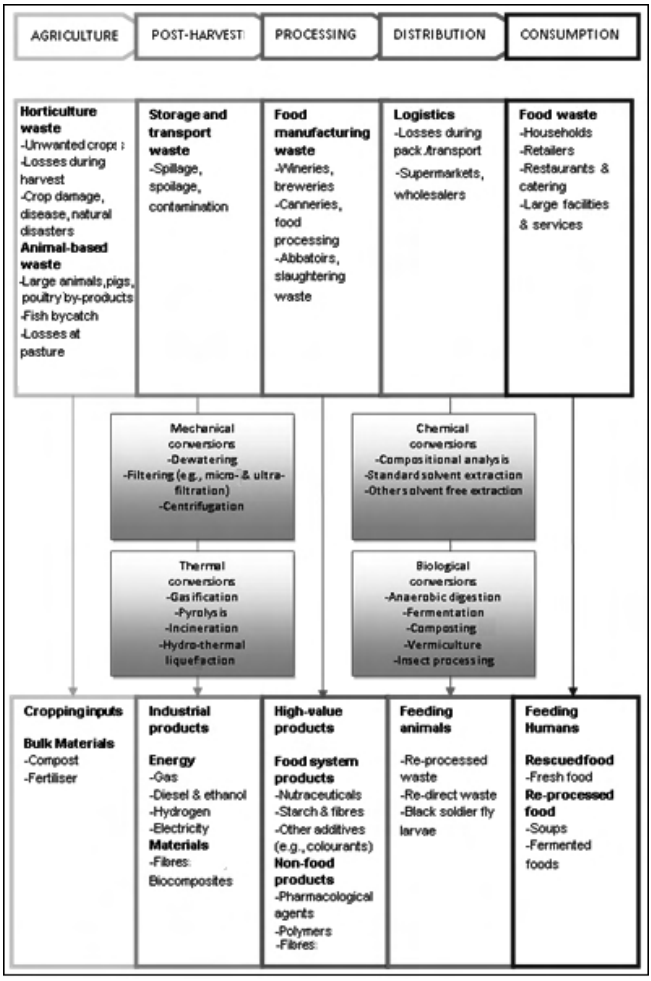
3.2 Reduction of food waste through circular economy practices

Since food waste is generated during primary production, food processing, wholesale and retail trade, food preparation, and consumption (*NALED*, 2019), there are opportunities for waste reduction, reuse, and recycling throughout the supply chain of agri-food products. *Fig. 2* underlines the possibilities of creating value from food waste by applying circular economy principles in the agri-food value chain.

According to *Pour* and *Makkawi* (2021), food waste management methods are classified into two main categories: disposal and recycling. Disposal is a method by which food waste is considered trash or, as of limited value, discarded without processing or pre-treatment. Recycling presents a management method applying collection and processing to upgrade the food waste to a value-added product, for instance, compost or biofuel. The methods of food waste recycling and other recovery, presented in *Fig. 2*, can be classified as follows: mechanical conversation such as dewatering, filtering, and centrifugation; chemical conservation such as transesterification – one of the most promising options for food waste recycling to biodiesel; thermal conservation such as incineration – matured waste treatment technology under the category of thermal or thermochemical conversion; biological conservation such as composting – the transformation of organic compounds to ammonia-nitrogen, carbon dioxide, or complex recalcitrant materials (also known as humic substances) under aerobic conditions; anaerobic digestion – the method used for the generation of methane from organic matter in the absence of oxygen; fermentation – the process that produces ethanol.

Since a large amount of waste is generated by households, we will deal with this type in more detail below. Based on the experiences of other countries and available professional and scientific literature on the management of the system and technological procedures of kitchen waste processing, *Fig. 3* gives a schematic presentation of kitchen waste flows, technological processing processes, and obtained products.

As shown in *Fig. 3*, sorting kitchen waste can be classified as producing biodiesel from edible waste oil, bioethanol, and animal feed from kitchen waste. Unsorted kitchen waste processing operations can be classified as anaerobic digestion, composting of kitchen waste, and thermal treatment, including incineration, pyrolysis, gasification, and hydrothermal carbonization. The final option for kitchen waste treatment is landfilling, the most undesirable option.



Source: reproduced from KPMG, 2020

Figure 2. CE approach to the agri-food value chain

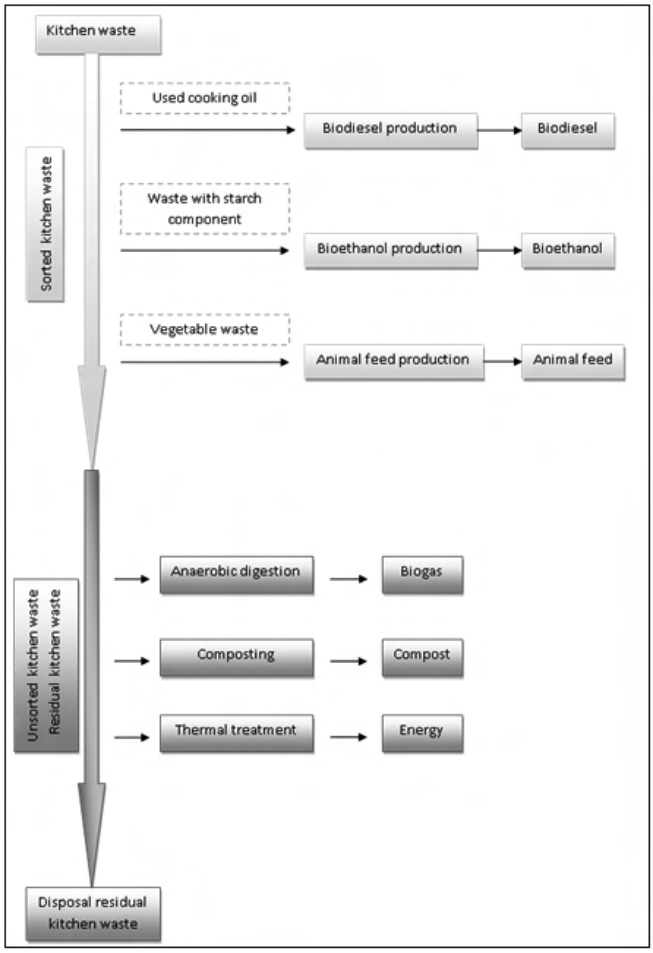
Given the enormous volume of waste, there has been much interest in developing new and novel waste recycling systems with properties like rapid, clean, sustainable, and cost-effective recycling. Therefore, the CE concept should be an obligatory waste management strategy to reach this goal.

In general, composting or biogas production from food waste is reasonably straightforward, with benefits in waste management, soil quality, air emissions, and resource use (*Kruchten & Eijk*, 2020).

Collecting and recovering 1,000 tons of food and organic waste, for example, would generate 60% more GDP and 40% more employment than disposing of it (*Karić et al.*, 2022).

3.3. Food waste as the cross-cutting issue of SDGs through the CE concept

The Post-2015 Development Agenda is built on the Sustainable Development Goals (SDGs), a set of 17 goals and 169 targets that must be met by 2030 (*UN*, 2015). However, the current formulation of SDGs does not recognize the importance of sustainable food waste management as a specific goal. Although there are no specific SDGs on food waste, many goals and related targets include direct, moderately direct, and indirect references to food waste. In addition, food waste is reflected in several goals and targets, such as health, cities, water, oceans, human settlements, and sustainable production and consumption. As a result, proper food waste management is a vital cross-cutting issue that can assist in resolving global and local problems (*Payet*, 2015).



Source: reproduced from NALED, 2019

Figure 3. Flows, processing and products from kitchen waste

SDG 2 "Zero Hunger" emphasizes extreme hunger and malnutrition, as these issues continue to be challenges for long-term development (less productive individuals, more prone to disease, and unable to earn more for improving their livelihoods) (*Kruchten & Eijk*, 2020). In SDG 2, the two targets are directly related to food waste, as presented in *Fig. 4*.

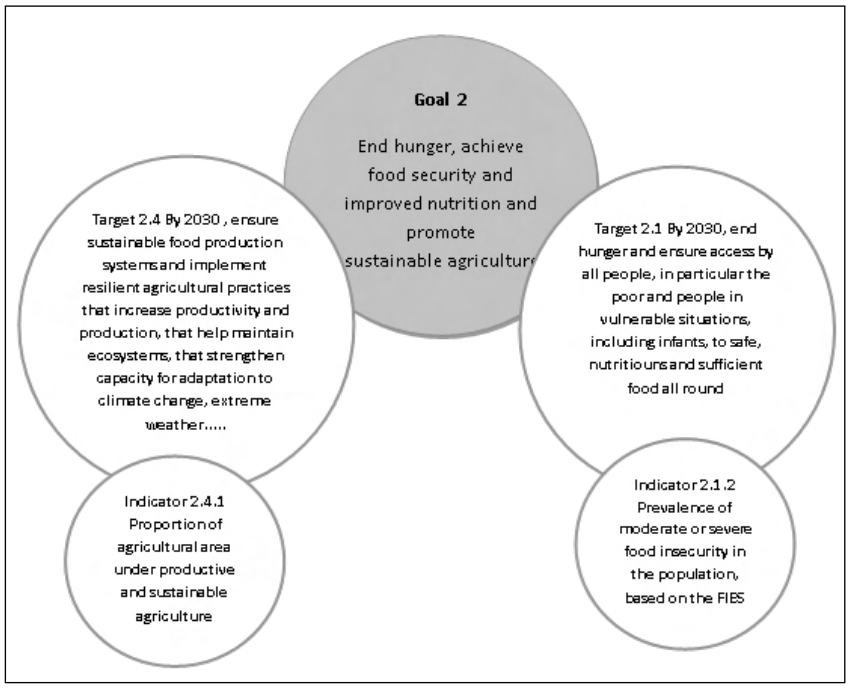


Figure 4. SDG 2 End hunger – directly related to food waste

One way to reach the targets of SDG 2 is by applying circular agriculture, which can help restore and improve soil quality by utilizing techniques, for instance, designing local nutrient loops. Furthermore, biomass from agriculture practice can be crucial for CE. For instance, biomass is a resource for food, animal feed, materials, transport fuel, and energy.

SDG 12, *Ensure Sustainable Consumption and Production Patterns*, aims to reshape consumption and production patterns to reduce resource pressure while promoting human and economic development (*UN*, 2015). On the other hand, unsustainable consumption and production patterns increase waste generation and the pressure on the environment and social and economic aspects of society. Diverse negative environmental impacts are caused by various extractive and processing industries and waste disposal, especially dumping and burning around urban areas.

In SDG 12, Member States also decided to – in target 12.3, "by 2030 [–] halve per capita global food waste at the retail and consumer levels and reduce food losses along production and supply chains, including post-harvest losses". SDG 12, with its 12.3 target, refers specifically to reducing food waste by 50%, which would allow a decrease of 20 to 30% of the total food-sourced GHGs (*Lombardi & Constantino*, 2021). The significant reduction of food loss and waste offers profound benefits, including avoiding environmental impacts generated across the supply chain for food ultimately discarded, often at the landfill, a crucial emitter of potent greenhouse gas, methane. Furthermore, food waste reductions in supply chains generate economic benefits related to efficiency gains. They may grow food availability on the global market (*Prokić et al.*, 2016).

In target 12.5, Member States decided to – "by 2030 [–] substantially reduce waste generation through prevention, reduction, recycling, and reuse". This target intends to reduce waste flows by taking a whole-life approach. It includes lowering overall resource input (addressed by other targets) and raising recycling and reuse rates, which aligns with the 3R method goals (reduce, reuse, and recycle). The target applies to industrial and household waste, including food waste. Therefore, it would help to minimize the quantity of waste landfilled and incinerated. Also, this target should help to reduce overall resource use by replacing primary resource inputs with recycled materials. In addition, establishing a recycling industry and collection system may add new employment (*UN*, 2015).

In SDG 12, the two targets are directly related to food waste, as presented in *Fig. 5.* Many of the circular practices specified in SDG 12 are vital, including water management, waste management, sustainable products and services, sustainable supply chains, and synergies with renewable energy. In addition, circular economy approaches can reduce the industrial pollution of water and soil where the circular 3Rs rule (reduce, reuse, recycle) is crucial to this problem. Food waste management is moderately directly related to SDGs 3, 6, 11, and 14. In SDG 3, target 3.9. covers a wide range of threats to human health. The quality of food waste management services can significantly impact environmental pollution and, subsequently, human contamination. However, the public and political profile of food waste management is often lower than other utility services (*Prokić et al.*, 2016). Unfortunately, statistics linking health outcomes with environmental pollutants from the food waste sector are generally poor (*Weitz et al.*, 2015).

In SDG 6, target 6.3 is a wide-ranging one. Groundwater worldwide is threatened by pollution from agricultural and urban areas, solid waste (including food waste), on-site wastewater treatment, and other industrial sources (SDG). In addition, dumpsites on land can cause pollution of both surface and groundwater (*Prokić et al.*, 2016). A CE can help access to adequate and equitable sanitation and hygiene for all by developing technologies and systems such as biogas systems (*Kruchten & Eijk*, 2020).

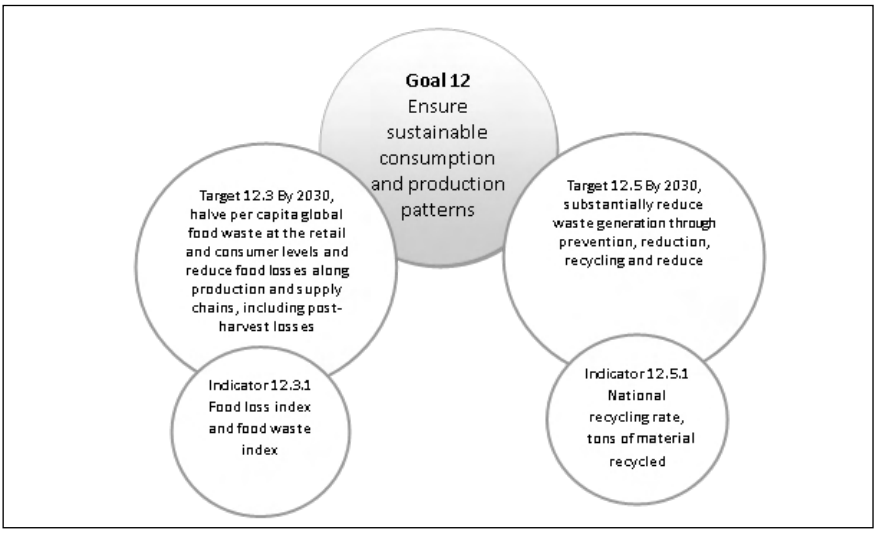


Figure 5. SDG 12 Responsible consumption and production – directly related to food waste

In SDG 11, Member States also decided to – in target 11.6. "by 2030 [–] reduce the adverse per capita environmental impact of cities, including by paying special attention to air quality and municipal and other waste management". A significant percentage of the population affected by pollution and waste lives in densely populated urban or semi-urban areas. Many cities produce more solid waste (including food waste) than they can dispose of properly. Even when municipal budgets are adequate for collection, the safe disposal of collected wastes remains a problem. Dumping and uncollected landfills are sometimes the main disposal methods in many developing countries; sanitary landfills are the norm in only a handful of cities. Solid waste management, including food waste, is essential for cities' sustainability, mainly if it includes: (food) waste reduction, reuse, recycling and composting, incineration, and disposal in landfills (*UN*, 2016). Core elements of circularity in urban areas are embedded within each critical urban system, from water, housing, and infrastructure to food and nutrition. Transition towards a circular city is a complex journey involving collaboration and coordination between the local government, businesses, organizations, technologies, and resources. Local governments are frequently confronted with the same question: what concrete steps can be taken to accelerate transitions towards a more CE? (*Kruchten & Eijk*, 2020). Goal 14 aims to conserve and sustainably utilize oceans, seas, and marine resources for sustainable development. In Goal 14, Member States also decided to – in target 14.1. "by 2025 [–] prevent and significantly reduce marine pollution of all kinds, particularly from land-based activities, including marine debris and

nutrient pollution". This goal is especially relevant if we consider the nutrient pollution from food waste.

However, almost every SDG could be linked to food waste management.

For instance, Goal 8, "promote sustained, inclusive and sustainable economic growth, full and productive employment and decent work for all", is linked to food waste management through recycling and resource recovery, activities that create green jobs and limit consumption of natural resources. Furthermore, Goal 15, "protect, restore and promote sustainable use of terrestrial ecosystems, sustainably manage forests, combat desertification, and halt and reverse land degradation and halt biodiversity loss", should be related to food waste management, considering that about 6% to 8% of all human-caused greenhouse gas emissions could be reduced if we stopped wasting food. All these facts indicate that food waste management is a cross-cutting issue impacting many aspects of sustainable development, and, considering food waste management issues, it needs to be included in every SDG through different, specific indicators.

4. Conclusions

A circular and sustainable economy is a prospective method for reducing food waste by implementing a production and distribution system in which food waste is reused or valorized into new products while reintegrating into market production channels.

Implementing a CE is a substantial task regardless of CE potential in food waste management. This task requires both macro- and micro-implementation and the vertical and horizontal integration of production and supply chains. Moreover, food waste management is a cross-cutting issue impacting many aspects of society and the economy. Therefore, food waste has strong linkages to SDGs. Although there is no specific SDG on food waste on the Post-2015 Development Agenda, several goals and related targets are directly, moderately directly, and indirectly related to food waste. Further, almost every SDG can be related to waste, considering waste as an environmental and economic and social issue.

The findings revealed that the strongest links and synergies between food waste management, circular economy practices, and SDG targets could be found in SDG 2 (Zero Hunger) and SDG 12 (Responsible Consumption and Production). Both goals have high scores for direct and indirect contributions. SDGs cannot be achieved without applying the concept of CE. Therefore, from a food waste perspective, a tremendous potential for the CE application is evident in the SDGs and targets directly related to food waste.

To implement the 2030 Agenda, the UN and other international organizations proposed specific waste management indicators for specific targets. Targets are

aspirational and global, with each government defining its national targets based on the global level of ambition but considering national circumstances. Each government should consider how these aspirational and global targets could be included in national planning processes, policies, and strategies, taking the circular approach into account.

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The Himalayan ethnic beverage tongba with therapeutic properties in high-altitude illnesses and metabolomic similarities to Japanese sake

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Abstract. Tongba, a millet-based fermented ethnic drink of the Limbu and other Nepalese-Tibetan communities, is consumed in the highlands of Singalila Ridge of the Himalayas and the adjoining high-altitude places of Nepal, the northern and north-eastern parts of India, and the Tibetan Plateau and is valued for its ethnomedicinal properties. In this research, the GC-MS-based metabolite profiling of an authentic sample of tongba was carried out, identifying various bioactive metabolites. Several biologically active components, such as glycoside, amino acids, fatty acids, and other long-chain hydrocarbon derivatives, terpenoids and phenol, were detected in tongba, which have therapeutic properties against various high-altitude illnesses. Probable biosynthesis routes of those compounds in tongba's broth were also studied, where many similarities were noticed with the Japanese beverage sake. The key finding of this metabolomic investigation was the detection of bioactive ethyl-α-D-glucopyranoside and cyclo(L-Leu-L-Pro) with abundant peak areas, which confirmed tongba's therapeutic importance in high-altitude illnesses and its metabolomic similarities with sake.

Keywords and phrases: traditional foods, ethnomedicine, fermented beverage, metabolomics

1. Introduction

An important portion of the world's population lives at high altitudes where daily lifestyle, food production, food availability, food requirements, and dietary habits are different and traditional, unlike in the rest of the world. According to biologist *Picón-Reátegui* (1978), food production and availability are definitely tied up to the altitude, weather conditions, soil, and other environmental factors of a region. A series of research has revealed how high altitude can influence human nutrition, dietary habits, and other activities of people (*Majumder et al.*, 2021). High-altitude ethnoecology shows a rational relationship between its people and their ethnic dietary habits, which directly or indirectly helps a group of people to endure the altitudinal stresses, including weather conditions (*Picón-Reátegui*, 1978). High-altitude dietary habits are distinct from the rest of the world, as these have been developed to help locals as well as tourists or visitors to get acclimatized to the surrounding environment, to stay well and recover from various high-altitude illnesses. Traditional knowledge on fermentation technology is considered an important part of food and beverage management in high-altitude regions where obtaining of important functional foods is very difficult and the preservation of food is required to increase the shelf life of processed or harvested foodstuff (*Majumder et al.*, 2021). The ethnoecology of Singalila Ridge of the Himalayas (the high-altitude place between Darjeeling, Sikkim, and Nepal) is very distinctive and enriched with plenty of traditional foods prepared by Nepalese and Tibetan ethnic communities, which include a lot of cereal-based fermented beverages such as: jand, or jaanr; raksi, or rakshi; nigar; chyang, or chhaang; tongba or tumba (*Tamang & Kailasapathy*, 2010; *Ray et al.*, 2016; *Majumder et al.*, 2021).

In several regions, finger millet grains are malted and fermented to obtain different traditional fermented drinks such as finger millet sake, one of the traditional millet-based fermented beverages that is consumed mainly in Japan and China. Tongba, or often called tumba, is one of the locally fermented millet-based traditional alcoholic beverages of the Limbu community of eastern Nepal, which is regarded a regular beverage by Tibetan, Nepalese, and other ethnic groups of people living in high-altitude regions in the Himalayas (parts of Nepal, northern and north-eastern parts of India, and the Tibetan Plateau) and is praised for its many ethnomedicinal properties, mainly for its anti-inflammatory or pain-relieving effects (*Tamang & Kailasapathy*, 2010). Tongba is prepared from brown finger millet (*Eleusine coracana*, also known as ragi in India or kodo in Nepal) grown in hilly regions, and it is cooked and combined with traditionally cultured khesung, which is a microbial colony or starter culture (*Tamang & Kailasapathy*, 2010). 'Khesung' is the Limbu version of the Nepali term 'murcha'; the Lepcha call it 'thamik', and Bhutias refer to it as 'phab'. Tongba is traditionally prepared in a round container and served in glass-shaped wooden or bamboo vessels after

pouring hot water over the fermented grains and sipped through a special bamboo straw with a perforated bottom that also functions as a filter (*Dangal et al.*, 2021). The resulting whitish liquor is thick and astringent, with a pleasant, mild flavour and distinctive taste. The vessel is refilled three to four times with hot water until the grains lose their potency (flavour, astringency, and taste) and the alcohol is exhausted. The term "tongba" actually means the bamboo vessel that holds the fermented millet beverage, which is traditionally referred to as 'mandokpenaa thee' (*Dangal et al.*, 2021). Traditionally, it is stored or aged for about six months, when the fermentation culture matures and flavours intensify and become mellower (*Harmayani et al.*, 2019).

Tongba, raksi, chyang, and jand, are all well documented in the field of ethnoecology along with some health claims, but only few of them (mainly raksi and chyang) have been subjected to foodomics to be studied scientifically at molecular level (*Majumder et al.*, 2021). So, the objective of this research was to study the volatile profile of tongba and metabolomics to understand the ethnomedicinal properties linked with the beverage. Analytical platforms like gas chromatography-mass spectrometry, liquid chromatography, capillary electrophoresis, nuclear magnetic resonance spectroscopy, etc. are commonly used in metabolomics research, among which GC-MS is believed to be the most used technique to date. GC-MS has also been tightly linked to foodomics since ages, where the technique is used to identify and quantify various substances of foods belonging to various metabolic pathways, including sugars, sugar alcohols, amino acids, fatty acids, and other organic acids, polyamines, terpenoids, alkaloids, etc. This research comprehensively provides a framework to facilitate the metabolomic evaluation of a traditional alcoholic beverage, and the outcomes would help to confirm the ethnomedicinal properties associated with the beverage.

2. Materials and methods

Sample collection

Six-month-old culture of traditionally brewed tongba (*Figure 1*) was collected from the oldest tavern of Kalipokhri village, one of the highest points of Singalila Ridge, situated at an altitude of 3,100 m near the Indo-Nepal border in the Darjeeling District of West Bengal. The sample collection site, Kalipokhri (27°04'45" N, 88°01'03" E), has been described as one of the oldest human settlements in Singalila National Park (*Majumder et al.*, 2021). An ethnobiological fieldwork was conducted in the traditional trekking routes of Singalila National Park (Dhotrey – Tonglu – Tumling – Gairibans – Kalipokhri – Bikheybhanjyang – Sandakphu – Gurdung – Srikhola) located in Darjeeling, India, and the Indo-Nepal border area to collect the authentic sample of tongba.

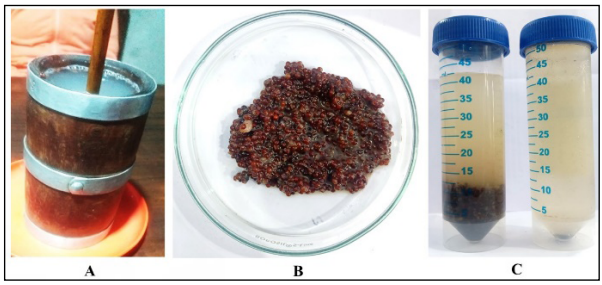

Figure 1. A – traditionally served tongba after pouring hot water over fermented culture, B – six-month-old culture of tongba, C – methanolic solution of the sample

Sample preparation for GC-MS analysis

The sample was collected in a sterilized container to avoid microbial contamination and kept inside the icebox before transferring it to the laboratory. Sample preparation for GC-MS analysis was done following the method standardized for alcoholic beverage analysis (*Majumder et al.*, 2021). A liquid portion (1 ml) from the sample of tongba was diluted in methanol (Merck) overnight to prepare the methanolic (50%) solution of tongba or TM (*Figure 1C*). Being a widely used and ideal solvent for the extraction of biochemicals, methanol was chosen. The polarity of methanol as an organic solvent also shows closeness with that of water or ethanol, which are primary components of alcoholic beverages (*Majumder et al.*, 2021).

GC-MS-based volatile profiling and metabolomics analysis

TM was subjected to GC-MS-based metabolomics following the standardized research protocol developed for the analysis of fermented beverages (*Majumder et al.*, 2021). GCMS-QP2010 Plus (Shimadzu Co., Japan) equipped with a DB-5 fused-silica capillary column (30 m × 0.25 mm × 0.25 µm) was used in this research. The analysis was performed by split-injecting (with a ratio of 20:1) 1 µl of TM. Injection temperature was 260°C, and interface temperature was 270°C. Ion source temperature was adjusted to 230°C. As carrier gas, helium (99.9%) was used. The total and column flow rate were 16.3 ml/min and 1.21 ml/min respectively. Mass spectra were recorded at the scanning rate of 5 scan/s. The compounds were identified after comparing the spectral configurations obtained with that of the available mass spectral databases, i.e. Wiley Registry of Mass Spectral Data, 8th Edition and The NIST 14 Mass Spectral Library (*Majumder et al.*, 2020). The chromatogram (TIC or Total Ion Chromatogram) was based on the intensity of fragments produced by the ionization. Quantification of the amount (area %) of

each compound was done based on peak areas. The data obtained from the GC-MS analysis were further studied based on available literature (*Majumder et al.*, 2022).

3. Results and discussions

The positive effect of tongba was reinforced by local inhabitants and brewers, who shared their knowledge on the ethnomedicinal properties of tongba as cardio-protective, pain reliever or anti-inflammatory, respiratory illness preventive, gastro-protective, etc. They also praised the drink for exhibiting potential moisture retention properties in the human body. Furthermore, the GC-MS metabolite profiling of this ethnic beverage was performed to determine its volatile composition and evaluate the claims of being therapeutic against high-altitude illnesses through studying the bioactivities of the metabolites that are described below.

The volatile profile of tongba and GC-MS-based metabolomics

A total of thirty-three peaks were found in the GC-MS chromatogram showing twenty-six different compounds (*Table 1*), where ethyl-α-D-glucopyranoside (α-EG) and cyclo(L-Leu-L-Pro) or (3S,8aS)-hexahydro-3-(2-methylpropyl)-pyrrolo[1,2-a]pyrazine-1,4-dione were found as major components occupying 53.95% and 16.96% of the total peak areas respectively. *Figure 2* includes a pie chart showing the percentage share of the types of metabolites based on chemotaxonomy and biosynthesis pathways involved in the broth of tongba (TM), where most of the glucoside has been found through the peak of α-EG. The sample also contained derivatives of terpenoids, fatty acids, and long chains of similar hydrocarbons, amino acids, phenols, sugar alcohol, antibiotic (actinomycin), alkaloid (dihydroergotamine), etc. (*Table 1*). The chromatograph is shown in *Figure 3*. Moreover, studying metabolomics has revealed various routes involved in the biosynthesis of those components whether they were directly derived from the substrate (finger millet) or due to fermentation, as metabolites of the khesung (traditional starter of tongba), which are described below.

Table 1. GC-MS peak report showing volatiles in TM

Name of compound	Area (%)	Type of compound
Tyrosol	2.72	Phenol
Ethyl-α-D-glucopyranoside	53.95	Glucoside
5-Methylcyclohexane-1,3-diol	0.37	Fatty alcohol
1,3-Methylene-D-arabitol	1.25	Sugar alcohol
Neophytadiene	0.42	Diterpenoid

Name of compound	Area (%)	Type of compound
Cyclo(L-Leu-L-Pro)	16.96	Cyclic dipeptide
Actinomycin C2	1.43	Cyclic peptide
14-Methyl pentadecanoate	0.36	Fatty acid
Ethyl palmitate	1.01	Fatty acid
Ethyl linoleate	1.47	Fatty acid
Methyl oleate	0.67	Fatty acid
Phytol	0.26	Diterpenoid
D,L-Pyroglutamic acid	0.32	Amino acid derivative
Ethyl linolenate	2.64	Fatty acid
Ethyl pentadecanoate	0.29	Fatty acid
3-Cyclopentyl-propionic acid, 2-dimethylaminoethyl ester	0.46	Fatty acid derived carboxylic acid
2,5-Di(trifluoromethyl) benzoic acid, 3-hexadecyl ester	1.05	Fatty acid derivative
Dihydroergotamine	2.7	Alkaloid
Phthalic acid	0.37	Chemical pollutant
Ethyl(dimethyl)(pentadecyloxy)silane	1.29	Fatty acid derivative
Arachidyl alcohol	1.96	Fatty alcohol
4-(2-Methoxyhexadecoxymethyl)-2,2-dimethyl-1,3-dioxolane	1.48	Fatty acid derivative
Farnesol	0.37	Sesquiterpenoid
β-sitosterol-methyl ether	0.85	Phytosterol
1-(2,3-Dimethoxypropoxy)-2-methoxyhexadecane	4.5	Long-chain alkane
β-Sitosterol	0.85	Phytosterol

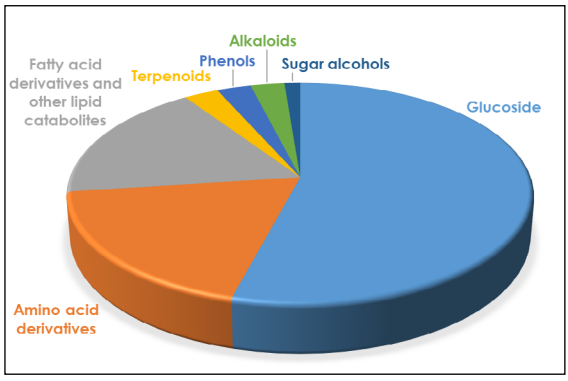

Figure 2. The metabolite types of tongba based on the GC-MS peak area

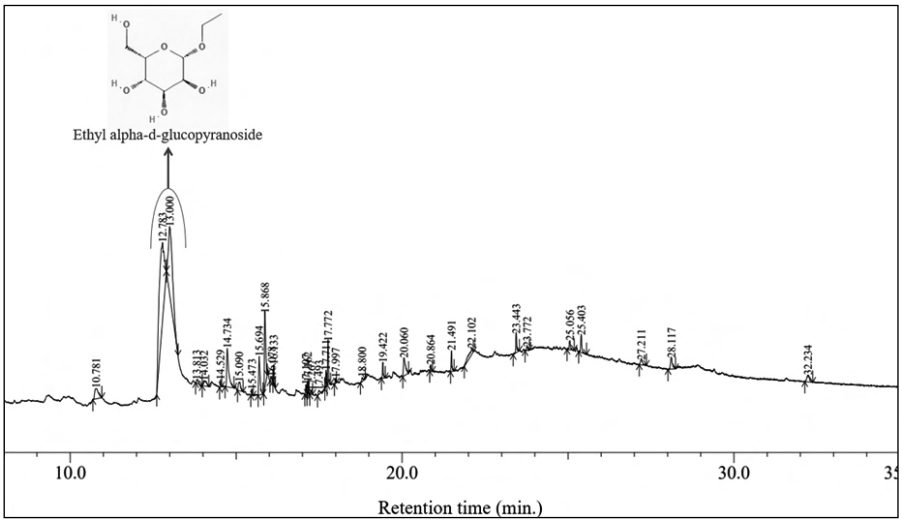

Figure 3. The GC-MS total ion chromatogram of the methanolic extract of tongba revealing the major compounds as α-EG (the compounds corresponding to peaks are listed in *Table 1*)

Glucoside

In tongba, the major component α-EG was detected with a total chromatographic peak area of 53.95%. This umami-flavoured compound is an abundant and typical component of sake, a Japanese traditional fermented beverage (*Bogaki et al.*, 2017). α-EG was later recognized as a safe and acceptable food component and also as one with beneficial biological activities on human health (*Mishima et al.*, 2005). Reports on the production of α-EG in sake by *Aspergillus oryzae* (*Imanari & Tamura*, 1971; *Bogaki et al.*, 2017) shed light on the probable biosynthesis pathway of α-EG in fermented broth, where α-EG is produced via starter-derived α-glucosidase activity due to the trans-glycosylation of substrate-derived maltose or any maltooligosaccharides. The α-EG's precursor maltose is a disaccharide derived from starch due to the amylase activity of amylolytic microbes present in tongba. Interestingly, the total starch content of finger millet/kodo (tongba's substrate) is about 59% (*Devi et al.*, 2014), which has been reflected in tongba's volatile profile, as the overall peak area occupied by starch catabolites (glucoside, sugar alcohol, and terpenoids) was 57.95%.

Amino acid derivatives

Three amino-acid-derived compounds detected in TM occupied a total of 18.71% of the area including peaks of major compound cyclo(L-Leu-L-Pro) (of 16.96% of total peak area), actinomycin C2, and DL-pyroglutamic acid. Cyclo(L-Leu-L-Pro), a

homodetic cyclic dipeptide composed of leucyl and prolyl residues, functions as a fermented metabolite (bacterial). The Japanese traditional fermented beverage sake, the distilled liquor awamori (*Yamamoto et al.*, 2016), and Himalaya's millet-based distilled liquor raksi (*Majumder et al.*, 2021) are reported to contain this major compound of tongba. It is a taste-modulating compound responsible for the characteristic taste and aroma of various fermented beverages such as beer, wine, and sake (*Yamamoto et al.*, 2016; *Acharyya et al.*, 2021). Pyroglutamic acid was also reported in different fermented foods such as sake and soya sauce (*Gazme et al.*, 2019), the Tanzanian traditional millet-based liquor togwa (*Mugula et al.*, 2003), and the Korean ethnic rice wine makgeolli (*Kim et al.*, 2008). *Gazme et al.* (2019) also demonstrated the biosynthesis of pyroglutamic acid in fermented sake due to the protein digestion by the protease enzyme of koji or *Aspergillus oryzae* (starter culture of sake). However, pyroglutamic acid is reported as a metabolite of finger millet (*Kim et al.*, 2013), the substrate of tongba. The pyroglutamic acid in the sample may be derived either from substrate or after fermentation as the breakdown products of proteins and peptides if metabolomics is considered. Another cyclic peptide, the actinomycin C2, is an antibiotic of bacterial (*Streptomyces* sp.) origin (*Saravana Kumar et al.*, 2014); so, the actinomycin in tongba-fermented broth is definitely a microbial metabolite.

Fatty acid derivatives and other lipid catabolites

Including the derivatives of long-chain hydrocarbons such as fatty acids, fatty alcohols, alkanes, and carboxylic acid derived from lipid breakdown, a total of thirteen such components of TM (*Table 1*) have been grouped together based on biosynthesis (lipid metabolism) and for possessing chemotaxonomic similarities. The occurrence of these components in TM causes no confusion, as the substrate kodo or finger millet contains lipids of about 1.3% (*Devi et al.*, 2014), and fermenting microbes also metabolize lipids to produce fatty acids and alcohols. Fatty acids such as linoleic acid and linolenic acid were previously reported as finger millet metabolites (*Kumar et al.*, 2016). Moreover, the fermented millet-based distilled alcohol raksi was also reported to contain palmitic acid and linoleic acid (*Majumder et al.*, 2021). *Majumder et al.* (2021) referred to those fatty acids of raksi as fermentation metabolome existing in various fermented beverages. The Japanese ethnic liquor sake also contains fatty acids, such as palmitic acid, linoleic acid, and linolenic acid (*Ishikawa & Yoshizawa*, 1979), which shows a metabolomic relationship between tongba and sake, in the same way as discussed above in relation to glycoside and amino acid derivatives. Arachidyl alcohol, palmitic or hexadecanoic acid, and pentadecanoic acid were already reported in finger millet sake (*Liu et al.*, 2015), which helped to validate this metabolomic study. The ethyl and methyl esterification of fatty acids is a common phenomenon during alcoholic fermentation, which reportedly contributes to the formation of distinct wine-like

flavour (*Yin et al.*, 2019). Earlier, *Ishikawa & Yoshizawa* (1979) described this same event in the fermentation of sake.

Terpenoids

Diterpenoid neophytadiene, its precursor phytol, sesquiterpene alcohol farnesol, and the phytosterol compound beta-sitosterol are components of the terpenoid biosynthesis pathway of TM (*Table 1*). Farnesol is reported as an important component of the Korean ethnic rice wine makgeolli (*Ha et al.*, 2014), which is also the precursor of various bioactive terpenoids (*Majumder et al.*, 2020), which metabolomically validates its presence as a fermented product of tongba. Phytosterol, beta-sitosterol, chlorophyll-derived phytol and its derivative neophytadiene are typical plant metabolites, where beta-sitosterol is specifically reported as a millet metabolite (*Islam et al.*, 2018). Thus, metabolomics suggested that these terpenoids in tongba were obtained directly from substrate.

Other components

Three other bioactive components with notable peaks were detected in TM, which are sole representatives of their chemotaxonomical categories; these are: tyrosol of the phenol, sugar alcohol – 1,3-methylene-D-arabitol, and dihydroergotamine of the alkaloid. The naturally occurring phenol tyrosol is a millet metabolite (*Sun,* 2017) and is documented as a millet fermentation product as well (*Ren et al.*, 2021). The production of tyrosol by *Saccharomyces cerevisiae* is reported in sake and the glutinous millet-based wine huanhjing (*Soejima et al.*, 2012; *Ren et al.*, 2021), which collectively validated tongba's similarity with sake and the occurrence of this compound in this millet-based beverage. The sweetener compound arabitol is a yeast metabolite and can be found in various fermented foods (*Kordowska-Wiater*, 2015). *Saha et al.* (2007) reported this compound as the main product of glucose fermentation by *Zygosaccharomyces* sp., starter of various fermented beverages, including kombucha. A series of research has revealed sugar alcohol arabitol as an important and typical component of sake besides amino acid derivatives and major glucoside compound α-EG (*Imanari & Tamura*, 1971; *Takenaka et al.*, 2000). Alkaloid dihydroergotamine is actually a secondary metabolite of ergot fungi, and, additionally, millet (substrate of tongba) is an important host of ergots (*Haarmann et al.*, 2009); therefore, metabolomics has confirmed this component as a substrate-derived contamination or microbial output.

The glycoside compound α-EG; amino acid derivatives, i.e. cyclo-D-Leu-L-Pro and pyroglutamic acid; fatty acid derivates; other compounds, i.e. phenolic tyrosol and sugar alcohol arabitol, etc. revealed a metabolomic similarity between tongba and sake. The biosynthesis of these fermented metabolites are dependent

on both substrate and starter material. It is known that millet is the substrate for both tongba and sake. On the other hand, starter cultures are also similar, comprising mixed cultures of fungi and bacteria with starchy cereals as the base. These starter cultures are usually found in the form of dried powder, flattened cakes, or hard balls of various sizes (*Koay et al.*, 2022). Both sake and tongba are fermented using amylolytic starter cultures usually containing amylolytic fungi for starch hydrolysis and yeast for alcohol production (*Koay et al.*, 2022). The spores of the *Aspergillus oryzae* (koji-kin) are added to the steamed rice, which is then incubated to produce koji, starter of sake. *Aspergillus oryzae* is a filamentous mould grown on rice (also known as koji mould), which is responsible for the production of most of the sake's metabolites but mainly of the major compound α-EG. Tongba's starter khesung or murcha is also prepared by pounding overnight-soaked glutinous rice (*Tamang & Kailasapathy*, 2010) that contains a microflora rich in the rice-grown amylolytic filamentous mould *Rhizopus oryzae* yeast species such as *Saccharomycopsis fibuligera*, *Saccharomyces bayanus*, *Candida glabrata*, *Pichia anomala*, *Saccharomycopsis capsularis*, and *Pichia burtonii*, and probiotic-candidate amylolytic lactic acid bacteria (*Tamang & Kailasapathy*, 2010; *Koay et al.*, 2022; *Olee et al.*, 2022). Therefore, the presence of rice-grown fungi in the rice-based starter khesung or murcha is natural, these fungi being responsible for the production of metabolites similar to those of sake. Just like *Aspergillus oryzae*, both *Rhizopus oryzae* and *Saccharomycopsis fibuligera* show glucoamylase, alpha-amylase, and protease activity (*Olee et al.*, 2022). Hence, the production of the starch breakdown product α-EG and the protein-derived cyclo(L-Leu-L-Pro) and pyroglutamic acid during the fermentation of tongba is acceptable. However, the microbiological assessment of tongba is yet to be performed, as besides this metabolomic interpretation, it is required to evaluate the biosynthesis pathways of α-EG and other compounds in tongba.

The bioactive compounds of tongba and their therapeutic effects in high-altitude illnesses

In highlands, moisture availability in the atmosphere decreases with elevation, where the human body, mainly the skin, must retain moisture on its own or by using supplementations. Reportedly, TM's major compound α-EG can have skin moisturizing and moisture retention effects and can decrease skin irritation also (*Bogaki et al.*, 2017). *Bogaki et al.* (2017) discovered α-EG's potential proliferation-activating effect on the fibroblasts of human dermis, which consequently increases the production of collagen, the most abundant protein of the human body (main structural protein in the extracellular matrix of connective tissues) to exhibit effective damage-repairing activities or speedy healing properties on various body parts, including the skin, tendons, ligaments, bones, and muscles. A formulation

enriched with α-EG would definitely be beneficial for humans in highlands, where a speedy healing of the skin, muscles, ligaments, and bones is required. Therefore, a traditional beverage like tongba, offering the same, should always be welcomed in high-altitude conditions. Earlier, *Mishima et al.* (2005) described α-EG as a diuretic component that increases urine production without affecting renal cells or functions, the blood glucose level, and insulin production. This biological activity can be cardioprotective, as frequent urination lowers high blood pressure.

Cyclo(L-Leu-L-Pro) is a potential antifungal metabolite that can also possess antiviral and antibacterial (*Zhao et al.*, 2021), antimutagenic, antifouling, antiprotozoal, and various similar properties of pharmacological importance (*Acharyya et al.*, 2021). Antibiotic actinomycin C2 is an antioxidant and displays cytotoxic activity against various human pathogens (*Saravana Kumar et al.*, 2014). Pyroglutamic acid possesses hepatoprotective, antidepressant, and anti-inflammatory properties (*Gazme et al.*, 2019). Similarly, the millet-based fermented beverage raksi is reported to exhibit biological activities against different diseases and disorders associated with altitude sickness due to the presence of such fatty acids (*Majumder et al.*, 2021). Fatty acid palmitate and linoleate are cardio-protective agents that can exhibit properties as vasodilators, antihypertensive and coronary heart disease preventives, which seems to be helpful in highlands (*Majumder et al.*, 2021). Palmitic acid and pentadecanoic acid have many other biological activities such as anti-inflammatory, antioxidant, and antibiotic (*Anyasor et al.*, 2015; *Venn-Watson et al.*, 2020; *Majumder et al.*, 2021). Linoleate is reported to prevent hypoxia and related respiratory illnesses and altitude-sickness-related symptoms such as fatigue, dizziness, vertigo, etc. (*Majumder et al.*, 2021). Moreover, even-chain fatty acid palmitate [C:16] and odd-chain pentadecanoate [C:15] are reported as beneficial for human health, and both have been detected in the sample TM including three derivatives of each (*Table 1*). Recently, *Venn-Watson et al.* (2020) have reported the efficacy of pentadecanoate in reducing cardiometabolic diseases, inflammation, anaemia, breathing problems, and chest pains causing disorders, pulmonary fibrosis. As major compound α-EG, straight-chain fatty alcohol and arachidyl alcohol also help to prevent skin damages by retaining moisture (*Lukic et al.*, 2021). Arachidyl alcohol is typically produced from arachidonic acid, which also exhibits a wide range of bioactivities, but mainly anti-inflammatory, cardio-protective, antidiabetic, antiviral, and antibacterial activity, and it can inactivate the severe acute respiratory syndrome (SARS-CoV-2) (*Das*, 2020). Beta-sitosterol, phytol, and neophytadiene are reported for their anti-inflammatory, antioxidant, and antimicrobial activities (*Bhandari & Lee*, 2013; *Islam et al.*, 2018). Phytol can exhibit neuroprotective properties such as anxiolytic and anticonvulsant effects (*Islam et al.*, 2018). Farnesol is a potential cardio-protective, antimicrobial, antitumor, antioxidant, antidiabetic, and neuroprotective compound (*Delmondes et al.*, 2020). This compound acts like a vasodilator and natural calcium channel

blocker to reduce hypertension and exhibits bioactivities against disorders directly linked to high-altitude illnesses such as asthma, edema, and inflammation (*Delmondes et al.*, 2020; *Chen et al.*, 2019). Tyrosol has a wide range of medicinal properties, i.e. antioxidant, anti-inflammatory, antiulcer, antimicrobial, antitumor, antiviral, anticancer, antidiabetic, and antiallergic activities (*Sun*, 2017). Having pharmacological effects, a considerable amount of dihydroergotamine can be acceptable in food (*Beuerle et al.*, 2012), especially in a high-altitude beverage, as it can prevent migraine disorders and is reported as effective pain reliever for people with cluster headache and very frequent migraine attacks (*Tfelt-Hansen & Koehler*, 2008).

4. Conclusions

The ethnobiology of Singalila Ridge and the adjoining high-altitude places of Darjeeling, Sikkim, and Nepal are rich in various traditional elements, which remains to be studied and explored. This metabolomic study revealed that tongba, containing the collagen-producing and moisture-retaining compound ethyl-α-EG, could heal damages occurred to the skin and other connective tissues due to exposure to high altitude. Inflammation and high altitude are closely associated, as body muscle inflammation, joint pains, peripheral edema (swelling of hands, feet, and face), etc. are common high-altitude problems. Interestingly, in Singalila and the neighbouring hills, drinks like tongba are served as healing beverages to get rid of pains. Here, metabolomics validated this property by revealing the presence of many anti-inflammatory compounds such as pyroglutamic acid, palmitic acid, pentadecanoic acid, phytol, neophytadiene, β-sitosterol, and tyrosol. Studies on the bioactivities of the metabolites of tongba and on the biosynthesis of those components have conclusively discovered that this ethnic beverage is metabolomically similar to sake and is a potential element of Himalaya's ethnoecology.

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The role of selenium in nutrition and the manufacturing of selenium-enriched milk

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Abstract. The role of selenium has increased after the discovery of the first seleno-enzyme in the human body. Selenium supports growth, the immune system, the reproductive organs, thyroid and muscle function, has an antioxidant effect, and protects against free radicals. The recommended daily intake of selenium for adults is 55 µg/day, which cannot be covered with food alone in selenium-deficient areas. That is the reason why we chose as our research topic the production of functional food – in this research: milk – in which the selenium level has been elevated naturally.

In our work, we supplemented six Holstein-Friesian cattle feeds with a daily 1, 2, 4, and 6 mg/individual organic selenium, and then we measured the selenium content by ICP-MS. With a selenium enrichment of 1 mg/day, a 60% increase in selenium content was achieved, as the selenium content of milk increased from 32.93 µg/kg to 52.79 µg/kg. With the 2, 4, and 6 mg supplementation, the milk contained 97.2, 182.69, and 231.31 µg/kg selenium respectively. The latter is seven times more than the selenium content of the control sample. We have developed a recommendation for the amount of naturally selenized milk that should be consumed for different age groups. In our opinion, milk with increased selenium content could contribute to improving the selenium status of the population.

Keywords and phrases: functional food, dairy, Se-milk, selenium-enriched yeast feeding, health preservation

1. Introduction

Selenium is an essential element for the normal function of the human body. It has been known since the first selenium-containing enzyme (glutathion peroxidase) was discovered in humans (*Flohé et al.*, 1973) to exert its antioxidant activity through selenium-containing enzymes, thus improving the immune function, protecting the organism against certain viruses (*Pecoraro et al.*, 2022), decreasing inflammatory processes, and reducing the formation of harmful free radicals (*Steinbrenner & Sies*, 2009). Its role is essential in the regulation of anti-inflammatory processes and the functioning of the normal hormonal system (*Karag et al.*, 1998). Without wishing to be exhaustive, it reduces the risk of developing cardiovascular diseases, rheumatoid arthritis, fatty liver, and insulin resistance and dyslipidemia associated with polycystic ovary syndrome (PCOS) (*Hajizadeh-Sharafabad et al.*, 2019; *Kanafchian et al.*, 2018; *Shidfar et al.*, 2018; *Weeks et al.*, 2012). Although the positive physiological effect of selenium appears to be indisputable, *Stranges et al.* (2007) pointed out in their study that the long-term use of high doses (200 µg/day) of selenium might promote the development of type 2 diabetes (T2D). However, *Steinbrenner et al.* (2022) stressed that the measurable selenium-containing markers may not be the cause but the consequence of diabetes. *Dias et al.* (2021) also clarified that selenium intake has no effect on the development of T2D.

In the human body, selenium is found in amounts of 10–15 mg; it accumulates mainly in the pancreas, spleen, liver, and the kidneys. The recommended daily intake is 55 µg for children and adults over 15 years and 60-70 µg for pregnant and lactating mothers (*Institute of Medicine*, 2000; *National Institutes of Health*, 2021). According to a report by a committee set up by the FAO, the IAEA, and the WHO, the maximum daily tolerable limit, or tolerable upper limit intake level (UL), is 400 µg selenium, above which symptoms of selenosis are to be expected (*WHO*, 1996). Symptoms of short-term high selenium intake include garlic smelling or metallic palate, hair and/or nail loss, while chronic selenium poisoning may include nausea, vomiting, diarrhoea, skin rashes, tooth discolouration, fatigue, and nervous system abnormalities (*National Institutes of Health*, 2021). However, this UL dose cannot be taken in Hungary with food alone.

Research has shown that the absorption of selenium from the digestive tract is limited, most of it being excreted in the urine, so its utilization is low (*Bendhal & Gammelgaard*, 2004). Selenium supplementation is needed in areas with insufficient selenium supply, e.g. in Hungary. This is achieved using organic forms of selenium such as selenomethionine and selenocysteine. The population has

two options: make up for the deficiency by using food supplements or consume foods with increased selenium content.

Foods contain varying amounts of selenium – Brazil nuts (*Bertholletia excelsa*) have the highest selenium content, which means 70–90 µg per piece (*Chang*, 1995). Our richest foods in selenium are seafood, fish, animal offal, meat and meat products, and dairy products (*Navarro-Alarcon & Cabrera-Vique*, 2008).

Dietary supplements have been available to the general consumers since the 1980s to complement vitamins and minerals with tablets and capsules. Commercially available selenium products contain inorganic selenite, selenate, and organic (yeast-bound) selenium (Se-methionine) (*Horacsek et al.*, 2006).

According to many, it is optimal to replace selenium with food because the absorption of organic forms of selenium and selenino-amino acids is better than that of inorganic ones (*Surai*, 2000).

Annual milk consumption has shown a slight upward trend in the recent years. According to the Milk Balance of the Hungarian Central Statistical Office, in 2019, this was 206.4 litres (*KSH*, 2021). Milk alone provides 6–10% of our daily selenium intake, making it one of the main sources (*Csapó & Csapóné*, 2002). Selenium added to the feed of dairy cows allows the milk to be fortified with selenium. Supplementation could be done with sodium selenite, selenocysteine, selenomethionine, or even selenium-enriched yeast (*Cobo-Angel et al.*, 2014). For animal feed, the organic forms should also be preferred because of the higher toxicity of inorganic selenium forms and the fact that some of them are excreted in the rumen and intestinal gases, urine, and faeces (*Bokori et al.*, 2003).

Many research groups investigated the effect of selenium added to livestock feed. A Spanish work group, *Azorín et al.* (2020) added the same dose of inorganic and mixed (inorganic/organic) selenium supplementation and found that they could reach higher selenium milk level with the mixed feed additive. *Stockdale et al.* (2011) made an experiment on two groups of Holstein-Friesian cows. They tested if pasture feeding and TMR feeding influences the selenium level of milk when the selenium supplement is added as pellets within the high range of daily 14.5–36 mg. The researchers found that the way of feeding (grazing or not) has no effect on the selenium level of milk, which is a better indicator of feed-based selenium intake than the selenium content of blood serum.

2. Materials and methods

Our feeding experiment was set up in a dairy farm on the northern edge of Hajdú-Bihar County (Hungary) with six Holstein-Friesian cattle. The cows were selected so that their milk production represented the average of the herd; thus, we were not looking for individuals with exceptionally high or low performance. The cows

were isolated in the "calving section" for the duration of the experiment, which ran from 29 October 2018 to 8 April 2019. The animals were calved at the end of October 2018, and their ages at the start of the experiment are shown in *Table 1*.

Table 1. Age of dairy cattle individuals in the experiment

Cattle	**1**	**2**	**3**	**4**	**5**	**6**
Age (year)	7	3	3	3	3	2
Nr. of calving	5	2	2	2	2	1

A premix was prepared for the feeding experiment, which contained cornmeal (Nagyhegyesi Takarmány Kft.) and selenized yeast (SelPlex-2300, Alltech Hungary Kft.). A dosing spoon containing 1 mg of selenium supplement (43.5 g) was prepared for dosing. The premix was thus administered to the daily ration once a day per animal according to the given dose (*Table 2*). The basic feed contained 0.7 mg (summer) of selenium at the beginning of the experiment and 0.6 mg (winter) of selenium from 1.12.2018.

Table 2. Daily selenium intake during the experiment

Week of experiment	**Selenium content of basic feed (mg)**	**Daily supplementation (mg)**	**Total selenium daily intake (mg)**
1–2	0.7	0	0.7
3–6	0.7	1	1.7
7–10	0.6	2	2.6
11–14	0.6	4	4.6
15–18	0.6	6	6.6
19–24	0.6	0	0.6

The amount of selenium premix was increased every 28 days from 1 to 2, 4, and then 6 mg Se/cow/day. Control samples were taken before the start of the experiment; selenium feeding was continuous until 25.2.2019, and then, ending the supplementation, a clearance study was continued for 6 weeks.

The selenium content was determined exclusively from milk, so animals were not slaughtered at the end of the experiment. Milk samples were taken individually at the time of milking with an automatic sampling unit connected to the milking machine and were also tested on an animal-by-animal basis, and the results were evaluated by dose and sampling time.

The measurements were performed with ICP-MS (Thermo Scientific X-1 Series 2) at the Institute of Food Science of the University of Debrecen after concentrated acid digestion. Samples were prepared by two-step digestion for selenium analysis. A sample of 2.00 ± 0.01 g each was weighed into the disruptor tubes. For pre-digestion, 10 cm^3 of concentrated HNO_3 was added to the samples, which were then allowed to stand overnight and then held in a block digester at 60°C for a duration of 30 minutes.

For the main digestion, 3 cm^3 of 30% H_2O_2 was added, and the samples were repeatedly placed in the block digester and kept at 120°C for 90 minutes. The samples were cooled to room temperature, made up to 50 cm^3 with deionized water, and filtered through filter paper (Filtrak 388).

MS Excel (Microsoft) was used for data processing and graphical representations, while SPSS 20.0 (IBM SPSS Statistics) was used to perform the statistical analysis of the experimental results.

3. Results and discussion

The results are shown in *Figure 1*, which shows the selenium content of milk by treatment average.

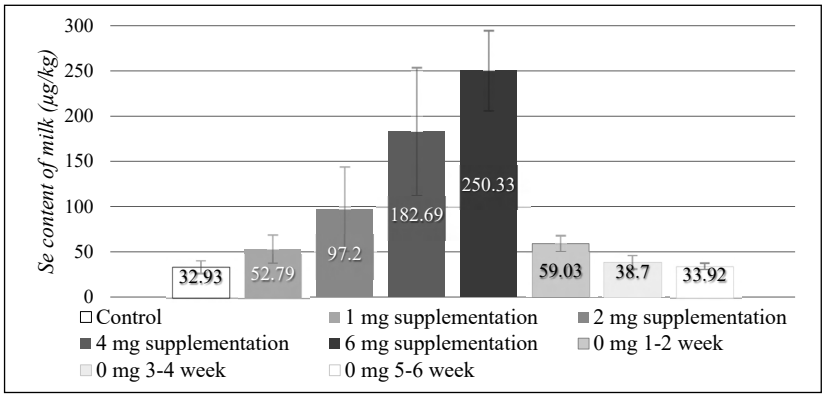

Figure 1. Changes in the selenium content of milk per treatment

The columns with the black numbers show the data when the cows did not receive selenium supplementation. The white-coloured column with black frame shows the result of the control sample. The sample taken at the beginning of the experiment contained 32.93 µg/kg selenium. As a result of the additions (3 grey bars with white numbers), the selenium content increased significantly in all cases due to the doses of 1, 2, 4, and 6 mg/cow/day, and treatment averages increased

from 52.79 µg/kg to 97.20 µg/kg, to 182.69 µg/kg, and then to 250.33 µg/kg. In the 19th week of the experiment, no more extra selenium was added, following which the selenium content of the milk decreased to the level of the control samples during the six-week monitoring period.

For consumers aiming to cover their personal daily selenium needs from milk alone, the graph included in *Figure 2* could be informative. Based on experimental results, the curve shows the recommended amount of selenium-containing milk with different selenium concentrations.

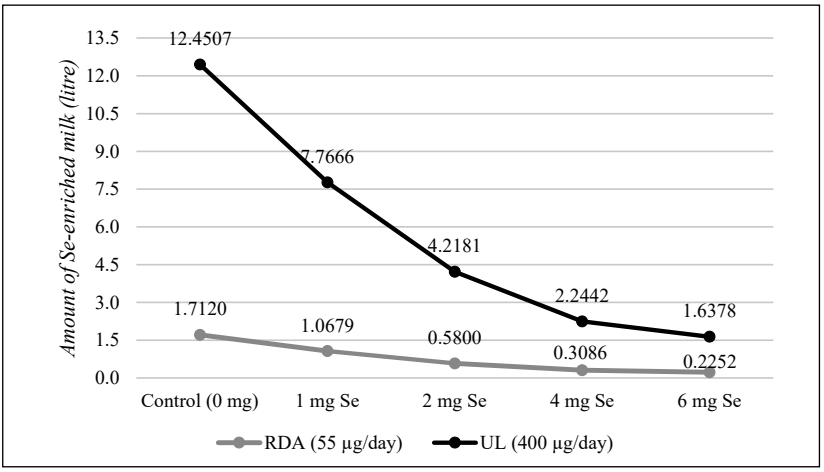

Figure 2. Selenized milk consumption recommendations for adults

The recommended daily intake for adults (according to WHO) is shown in grey, and the upper limit is indicated in black. The average milk consumption of the population cannot be modelled, and selenium is also absorbed from other sources, wherefore we recommend the marketing of milk obtained with a 2 or 4 mg supplement (Se_2-milk and Se_4-milk) for health protection purposes.

As selenium binds to milk protein, the selenium content does not decrease during fat adjustment and lactose removal, so there is no need for selenium replacement during industrial use, and fermented milk products and cheeses can be made from Se-milk. Due to the very high selenium content of Se_6-milk, it is not recommended for industrial use due to the possible accumulation of selenium in dairy products.

Figure 3 shows the recommended intake for milk at 2 mg/day supplementation by age. The recommended amount calculated for Se-milk for infants aged 1 to 3 years is marked in black, which is equivalent to 210 ml, i.e. 1 glass of milk, at the recommended intake of 20 µg/day. Preschool and elementary school children (marked in medium grey) need a daily selenium requirement of about 320 ml

of milk, while primary school 5–8th grade students (10–14 years; shown in light grey) can cover their daily selenium needs with 420 ml of milk. For those over 15 years of age (marked with white column on the diagram), the intake value is 55 µg/day, which equals that of the adults and means almost 600 ml of Se_2-milk. In practice, this could be exploited if students in the school milk programme could receive 200 ml of Se-milk or cocoa in order for the beneficial effects of selenium to be felt from an early age.

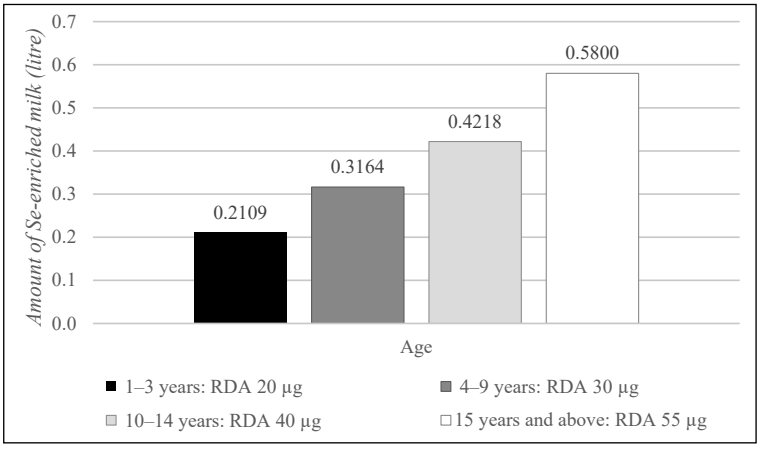

Figure 3. Se_2-milk consumption recommendations for different age groups

4. Conclusions

In our opinion, the experiment was successful because we were able to produce milk with elevated selenium level by feeding dairy cows. With 1 mg of selenium supplementation, the selenium level in milk increased by 60% from 32.93 µg/kg to 52.79 µg/kg. With doses of 2 and 4 mg/cow/day, the milk contained an average of 97.20 µg/kg and 182.69 µg/kg of selenium, which is more than 5.5 times that of the control sample. With a dose of 6 mg/cow/day, milk selenium levels increased to 702% of the original selenium content (to 231.31 µg/kg).

Although the research and clinical trials mentioned in the literature suggest an intake of Se above the RDA in order to maintain health and alleviate certain diseases, in our opinion, the optimal dose for Se supplementation would be 2 to 4 mg/day/cow for direct consumption in the case of healthy adults. In that case, one can take in the daily RDA level of selenium with ~ 3 to 6 dl milk without the risk of overdose [according to the UL, the amount of milk for chronic selenium overdose would be 2.24 litres (4 mg supplementation) and 4.22 litres (2 mg suppl.)]. For dairy production, we suggest using the 2 mg/cow/day selenium dose.

Evaluating the results, we believe that milk with increased selenium content is also important from a food science and health point of view, and as an essential food, it can be one of the cornerstones of adequate selenium intake and healthy eating.

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Assessment of sodium chloride tolerance and antibiotic resistance of *Citrobacter braakii* EC-PS1 and *Macrococcus caseolyticus* Li-PT1 isolated from artisanal cheeses

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Abstract. The different varieties of cheese are fermented dairy products with high nutritional value, which have been part of the healthy human diet for centuries. Cheeses are characterized by complex and diverse microbiota carrying beneficial, spoilage, or foodborne pathogen bacteria. Harmful bacteria originate from the production chain. Identification and characterization of microorganisms in cheese are important nutritional, food safety, and technological issues. During this study, *Citrobacter braakii* EC-PS1 and *Macrococcus caseolyticus* Li-PT1 bacteria strains, isolated on selective mediums from two artisanal cheeses, were identified by way of 16S rDNA gene sequence analysis. Their antibiotic resistance and NaCl stress tolerance were also determined. *M. caseolyticus* Li-PT1 showed tolerance to 6.5% and 10% NaCl. The increasing NaCl concentration above 15% contributed to the decrease of viability in bacteria. The increasing NaCl concentration to 10% contributed to the decrease of viability in *C. braakii* EC-PS1 bacteria. The identified bacterial species exhibited different levels of resistance to the tested antibiotics. *C. braakii* EC-PS1 showed resistance to chloramphenicol, ampicillin, and streptomycin, whereas *M. caseolyticus* Li-PT1 was susceptible only to two antibiotics (erythromycin, tetracycline) out of the eight tested.

Keywords and phrases: osmotic stress, antibiotic resistance, cheese, food safety

1. Introduction

Tolerance to food-processing-, production-, or storage-associated stress conditions contributes to the survival of bacteria in adverse environmental circumstances. Foodborne pathogens (*Salmonella* spp., *Escherichia coli*, *Campylobacter jejuni*, *Listeria monocytogenes*) are able to adapt effectively to stressful conditions (*Bucur et al.*, 2018; *Oh et al.*, 2019; *Kim et al.*, 2021; *Guillén et al.*, 2020; *Guillén et al.*, 2021; *Duze et al.*, 2021).

The bacterial cells sense the stress on molecular level and respond to these conditions with specific stress responses, which enable bacteria to respond to particular suboptimal conditions in their immediate surroundings or with general stress responses (*Begley & Hill*, *2015*).

Some stress factors, including heat treatment, cooling/freezing, acidic conditions, or osmotic tolerance, may induce in several bacteria (*L. monocytogenes*, *E. coli*, and *Salmonella* spp.) the so-called cross-protection phenomenon (*Begley & Hill*, 2015; *Chen*, 2017).

Osmotic stress causes physiological or morphological changes in the cell of foodborne pathogens, inhibiting their growth or leading to cell death. The mitigation of the adverse effects of osmotic stress includes the modification of membrane proteins and transportation systems. The resistance to high osmotic habitats is rare in bacteria. These stress conditions contribute to the alteration of the osmoregulation of the cell resulting in the deterioration of metabolic activities (*Ding et al.*, 2022).

Safe food production implies that foodborne pathogens are exposed to high osmolarity conditions. Many bacteria species in these environments respond with persistence and adaptation regarding the morphology, growth dynamics, or biofilm development (*Sleator & Hill*, 2001; *Ding et al.*, 2022).

The reply of the cells consists in the accumulation and release of osmolytes as organic molecules (trehalose, proline, trimethylammonium compound, glycine betaine, and carnitine) or inorganic ions (K^+) through active transport. In case of high osmolality, the compatible compounds prevent dehydration of the bacterial cell. The accumulated osmoprotectants alter minimally the physiology and cellular functions. Different factors are involved in response as transporters, multiple enzymes and channels with redundant specificities and functions (*Sleator & Hill*, 2001; *Wood*, 2015; *Bremer & Krämer*, 2019; *Ding et al.*, 2022).

Reaction to osmotic challenges in Gram-positive and Gram-negative bacteria is via potassium ion uptake stimulation, which contributes to stabilizing the net negative charge of the macromolecules in the cytoplasm (*Sleator & Hill*, 2001). The mitigation of hyperosmotic stress was associated with lysine-to-α-aminoadipic-δ-semialdehyde pathways in *Silicibacter pomeroyi* (*Neshick et al.*, 2013). In *Streptomyces coelicolor,* several transcriptional regulations of enzymes are involved in the alleviation of stress conditions (*He et al.*, 2018).

Growth in high-salt media implies the modification of cytoplasmic membrane fatty acid compositions, related to the phospholipids' head group, too. It has been shown that *E. coli* respond to osmotic stress with an electrolyte due to growth in the proportion of cardiolipin and to the reduction of phosphatidylglycerol content. In non-electrolyte-induced osmotic stress, the phosphatidylglycerol content was not altered (*Romantsov et al.*, 2009). In *Enterococcus* spp. strains, the changing sodium chloride (NaCl) concentration (4%, 7%) altered the expression level of genes associated with virulence factors (*Zarzecka et al.*, 2022).

The different types of cheese belong to the group of fermented dairy products, which have been part of healthy human diet for centuries, containing essential nutrients and bioactive substances. Cheese consumption in many countries has increased significantly in recent years. In the European Union, about 36% of milk is used for cheese production (*de Oliveira et al.*, 2017). Cheeses are characterized by complex and diverse microbiota carrying beneficial, spoilage, or foodborne pathogen bacteria. The harmful bacteria originate from the production chain. Cheese is considered a valued product providing beneficial or harmful health effects. The negative effect is associated with the presence of foodborne pathogens. Raw milk cheese can be a source of human pathogenic bacteria such as *Salmonella* spp., *Streptococcus* spp., *L. monocytogenes*, *Helicobacter pylori*, *Campylobacter* spp., *Escherichia coli*, *Coxiella burnetti*, *Mycobacterium* spp., *Brucella* spp., *Staphylococcus aureus*, *Arcanobacter pyogenes*, *Bacillus cereus*, *Leptospira*, *Clostridium* spp., and *Yersinia enterocoliticia*. Unsuitable cheese-making conditions favour the growth and development of these species (*O'Sullivan & Cotter*, 2017).

The fermented foods without heat treatment could be a source of antibiotic-resistant bacteria. The uncontrolled use of antibiotics in dairy cattle contributed to the spread of antibiotic-resistant bacteria. *Staphylococcus* strains found in Minas Frescal cheese were shown to exhibit resistance to penicillin, oxacillin, and clindamycin and harbour antibiotic resistance genes such as blaZ, mecA, lsaB, msrA, and ant4 (*da Silva Abreu et al.*, 2020).

Different bacteria species belonging to the *Enterococcus* genus, such as *E. faecium*, *E. faecalis*, and *E. durans*, were detected in Protected Designation of Origin (PDO) cheese from six cheese-making units. Several bacteria strains showed antibiotic resistance to β-lactams, aminoglycosides, glycopeptides, quinupristin-dalfopristin, teicoplanin, and tetracycline (*Rocha et al.*, 2022).

Infections were caused by *Salmonella* species, including multi-resistant serotypes originated from cheeses (*Cogan*, 2011; *de Oliveira et al.*, 2017). Raw milk or raw milk cheese contaminated by enterotoxigenic *Staphylococcus* spp. can be considered in many cases the source of staphylococcal enterotoxin. In Brazilian cheese samples, they identified bacteria showing resistance to clinically relevant antimicrobials such as penicillin, cefoxitin, oxacillin, clindamycin, erythromycin, tetracycline, tobramycin, gentamicin, ciprofloxacin, chloramphenicol, and also

community-acquired methicillin-resistant *Staphylococcus aureus* (*Aguiar et al.*, 2022). Methicillin resistance was found in coagulase-negative staphylococci isolates and in *Macrococcus caseolyticus* (*Klempt et al.*, 2022). *M. caseolyticus* with casein-hydrolysing capacity was found in the bacterial community of cow's milk in artisanal cheeses from north-western Argentina (*Suárez et al.*, 2020).

Several studies reported that cheese produced from raw and heat-treated milk was associated with foodborne outbreaks caused by infection of *L. monocytogenes*, *S. aureus*, *Salmonella* spp., Shiga-toxin producing *E. coli*, *Campylobacter* spp., *Brucella* spp., *Shigella* spp., *Clostridium perfringens*, and *Bacillus cereus* (*van Asselt et al.*, 2017; *Adhikari et al.*, 2018).

Bacterial growth and development in various cheese types are controlled by different environmental factors (water activity, pH, ripening temperature, redox potential), composition (NaCl content, nitrates), metabolites, and bacteriocins. There are few studies on the prevalence, stress tolerance, and antibiotic resistance of spoilage and foodborne bacteria in locally available dairy products. The aim of the present study was the determination of the antibiotic resistance and NaCl stress tolerance of *Citrobacter braakii* EC-PS1 and *Macrococcus caseolyticus* Li-PT1 originated from cheese.

2. Materials and methods

During the study, the microbial contamination of salty type soft cheese and fresh cheese originated from open air markets, with cultivation methods on different selective media (ChromoBio TBX, Listeria Mono Differential Agar Base), was determined. Bacterial colonies with the highest number and with a characteristic colony morphology were isolated, and pure cultures were obtained. Identification of isolated bacteria strains was done using 16S rDNA gene sequence analysis (*György et al.*, 2021).

The antibiotic susceptibility testing of the isolated bacteria was performed with the disk diffusion method, according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines. A total of eight different antibiotic disks containing the antibiotics erythromycin 15 µg, chloramphenicol 30 µg, kanamycin 30 µg, ampicillin 10 µg, clyndamicin 2 µg, streptomycin 10 µg, tetracycline 30 µg, and gentamicin 10 µg were used (*György et al.*, 2021).

The tested bacterial species were grown for 28 hrs at 37°C and inoculated in nutrient broth with 2%, 4%, 6,5%, 10%, 15%, 20%, and 25% NaCl content. The optical density values were recorded at the wavelength of $\lambda = 595$ nm by Fluostar Optima Microplate Reader (BMG Labtech, Ortenberg, Germany) in every 4 hrs. Growth curves were obtained from the absorbance values. Bacterial survival rate was calculated according to *Nath et al.* (2020) with slight modifications.

3. Results and discussion

The emergence of antibiotic-resistant and NaCl stress-resistant foodborne pathogens has created great pressure on the food industry during processing and storage. The two identified bacterial species were isolated on the selective mediums ChromoBio TBX and Listeria Mono Differential Agar Base from salty type soft cheese and also from fresh cheese samples.

According to the results of the 16S rDNA sequence analysis, one of the bacterial isolates belongs to the *Macrococcus* genus, *M. caseolyticus*, previously classified as *Staphylococcus caseolyticus* and showing 99.27% similarity, whereas the other bacteria belong to *Citrobacter genus, C. braakii* EC-PS1 with 99.6% similarity.

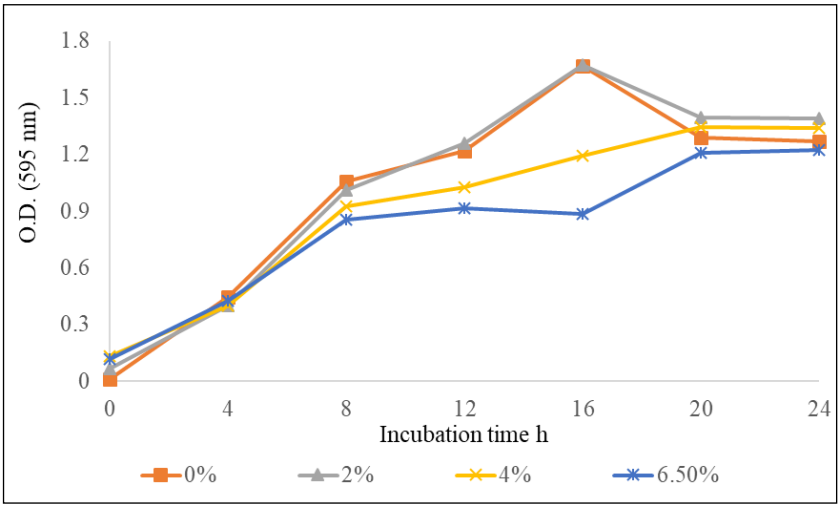
The *Macrococcus* genus taxonomically belongs to the family of *Staphylococcaceae* and comprises eleven species as follows: *Macrococcus bovicus*, *M. carouselicus*, *M. equipercicus*, *M. brunensis*, *M. hajekii*, *M. lamae*, *M. goetzii*, *M. epidermidis*, *M. bohemicus*, *M. caseolyticus*, and *M. canis.* Several studies reported isolation and identification from dairy (goat coalho cheese, Amazonian artisanal cheeses, raw milk) and meat products (fermented liver sausage). Alkaline-fermented foods with high protein content were also a source of *M. caseolyticus.* This is not considered a human pathogen but a relevant harmful bacteria causing infections in veterinary medicine (*Martins et al.*, *2018*; *Mazhar et al.*, 2018; *Ouoba et al.*, 2019; *Ribeiro-Júnior et al.*, 2020; *Arãgao et al.*, 2022; *Belleggia et al.*, 2022).

The Gram-positive bacteria were commonly isolated from commercially available animal meat and characterized with susceptibility to photocatalytic disinfection by nano-TiO_2 (*Wang et al.*, 2014). The insect products as meat alternatives originated from Belgian markets contained *M. caseolyticus* besides *E. faecium* (*Geeraerts et al.*, 2020). The nitrite content of milk powder was associated with the presence of thermophilic bacteria. Milk from a dairy processing plant was a source of *M. caseolyticus* with nitrate-reducing ability (*Wong & Flint*, 2019). *M. caseolyticus* was also a dominant species of bacterial flora of smoked fish (*Maïworé et al.*, 2021).

The facultative anaerobic Gram-negative *Citrobacter* spp. belongs to the family *Enterobacteriaceae* (*Liu et al.*, 2021). *Citrobacter braakii* was detected also by others in cheese samples. The predominant species of ewe milk and curd contained *C. braakii*, *C. freundii*, and *Klebsiella oxytoca.* The gas production of *C. braakii* was associated with early blowing in soft and semi-hard ewe cheeses (*Tabla et al.*, 2016). Examination of microbiota of the sheep cheese "bryndza" detected the presence of *C. braakii* (*Kačániová et al.*, 2019).

M. caseolyticus Li-PT1 growth was stimulated with 2% and 4% NaCl content, and the bacterial survival rate exceeded 100%. *M. caseolyticus* Li-PT1 showed tolerance to 6.5% and 10% NaCl (*Fig. 1*), with 96.07% and 64.71% survival rate. Increasing the sodium chloride concentration above 15% contributed to the decrease

of viability in bacteria – the rate of survival was 49.56%. The identified bacteria strain also showed reduced growth values at 20% and 25% NaCl concentration, with survival rates of 40.83% and 47.92%.



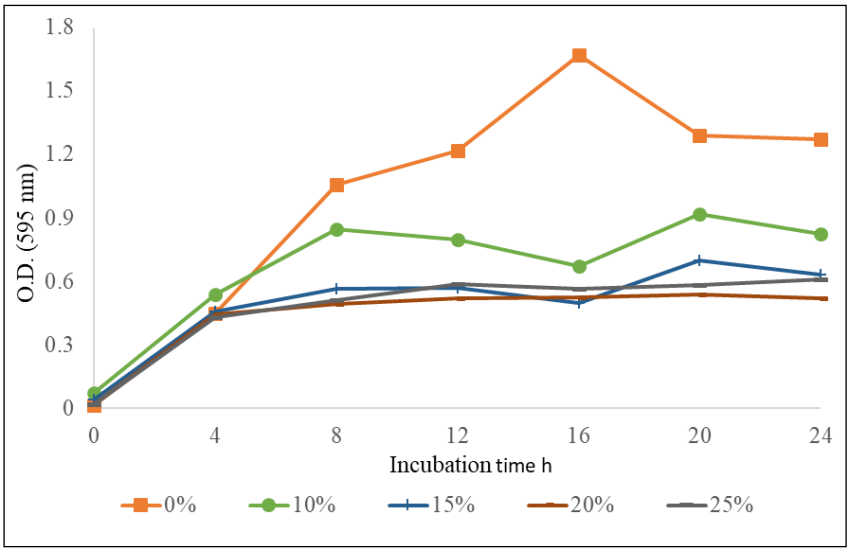


Figure 1a–b. Growth curves of *Micrococcus caseolyticus* Li-PT1, grown in media containing different concentrations of NaCl

The growth of *C. braakii* EC-PS1 was repressed by increasing the NaCl concentration of the growth medium (*Fig. 2*). As mentioned in the case of *M. caseolyticus* Li-PT1, *C. braakii EC-PS1* showed tolerance to 4% NaCl.

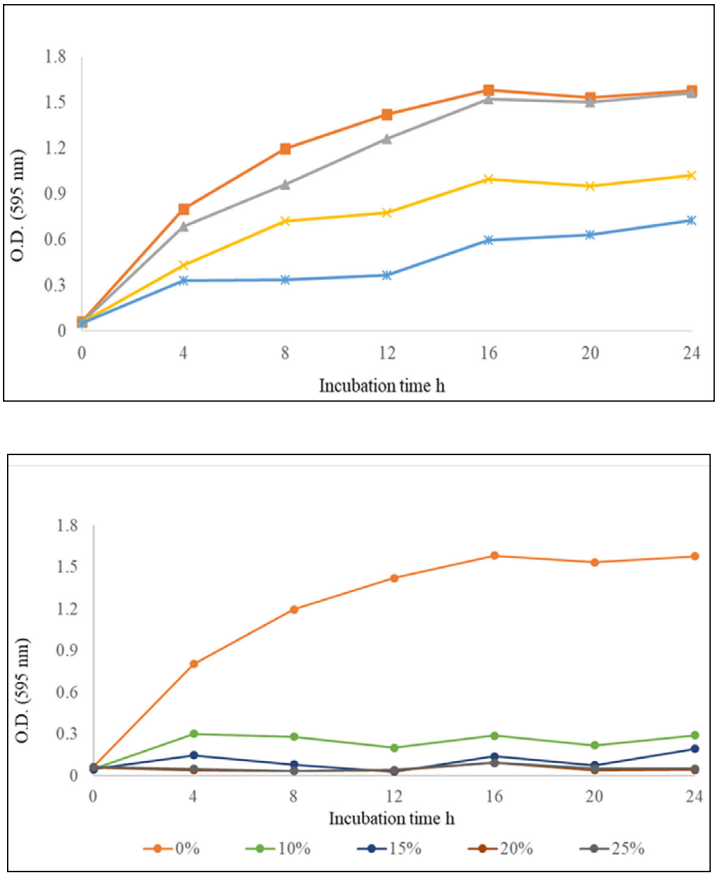


Figure 2a–b. Growth curves of *Citrobacter braakii* EC-PS1, grown in media containing different concentrations of NaCl

Increasing the NaCl concentration to 10% contributed to the decrease of viability in *C. braakii* EC-PS1 bacteria. These are also confirmed by the bacterial survival rate, which is equal to 99.02% in the presence of 2% NaCl, 64.68% in the presence of 4% NaCl, and 45.92% in the presence of 6.5% NaCl. The identified bacteria strain showed a minimal sign of growth at 10% NaCl, with a bacterial survival rate of 18.47%, and no sign of growth after 4 hrs of incubation above 10% NaCl.

Table 1. The antibiotic resistance pattern of the two identified bacterial species originating from dairy products

	Erythromycin 15	**Chloranphenicol 30**	**Kanamycin 30**	**Ampicillin 10**	**Clindamycin 2**	**Streptomycin 10**	**Tetracycline 30**	**Gentamicin 10**
Citrobacter braakii EC-PS1	N	R	S	R	N	R	S	I
Macrococcus caseolyticus Li-PT1	S	R	R	R	R	R	S	R

The identified bacterial species exhibited different levels of resistance to the tested antibiotics. *C. braakii* EC-PS1 showed resistance to chloramphenicol, ampicillin, and streptomycin (*Table 1*). *M. caseolyticus* Li–PT1 was susceptible only to two antibiotics (erythromycin, tetracycline) out of the eight tested.

The safety evaluation of microorganisms in the food chain represents a priority. One of the main public health concerns is the possibility of the resistance plasmids' transfer through the food chain to pathogenic and/or commensal bacteria, as in the case of *M. caseolyticus.* A whole-genome analysis showed multidrug resistance and methicillin resistance determinants in *M. caseolyticus* (*Mazhar et al.*, 2018). *M. caseolyticus* harbours different genes conferring resistance, such as mecA, mecB, mecC, and mecD. It has been demonstrated that bacteria exhibit gene-sharing ability between species.

Our findings regarding origin and antibiotic resistance are in concordance with other studies. *M. caseolyticus* from cheese samples showed resistance to cefoxitin, penicillin G, harbouring also methicillin resistance genes. Results suggest that this is a food safety concern because of the broad-host dissemination of the mecD gene (*Klempt et al.*, 2022). In milk samples originating from cows with subclinical mastitis, it was detected *M. caseolyticus* bacteria showing antibiotic resistance towards ampicillin, cefoxitin, erythromycin, oxacillin, and penicillin (*de Oliveira et al.*, 2022).

It was found that Gram-negative *C. braakii* rarely causes infections in relation to *C. freundii* and *C. koser.* Despite this, *C. braakii* was associated with bacteremia in a cervical cancer patient and with infections in immunocompromised patients (*Hirai et al.*, 2016; *Oyeka & Antony*, 2017), and it was isolated also from a patient treated with intravenous antimicrobial therapy (*Fernández-Polo et al.*, 2021). Regarding antibiotic resistance, *C. braakii* isolated from patients with extraintestinal infections was characterized as multidrug-resistant (*Liu et al.*, 2021).

4. Conclusions

The presence of antibiotic-resistant bacteria with stress tolerance in food production is a food safety concern. The tested dairy products contain antibiotic-resistant bacteria. The results obtained indicate that one bacterial strain could grow in sodium chloride stress conditions. Further investigation is necessary to reveal molecular responses of the bacterial strains to stress factors to prevent or control their growth.

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