Impact of Short-Time Micronization on Structural and Thermal Properties of Sugar Beet Fibre and Inulin

Running head: Sugar beet fibre and inulin properties affected by micronization

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SUMMARY

Research background. By tailoring dietary fibres’ structural and physicochemical properties, their functionality and applicability can be remarkably increased. One of the approaches used in this respect is fibre particle size reduction. Accordingly, the present study explores the impact of short-time micronization in a planetary ball mill on structural and thermal changes of modified and commercial sugar beet fibres, inulin, and sucrose in terms of their potential application as food excipients.

Experimental approach. Short-time micronization in a planetary ball mill (30 and 60 min) was applied for particle size reduction of modified and commercial sugar beet fibres, inulin, and sucrose as less energy consumptive and less destructive approach compared to long-time micronization. Dietary fibre and sucrose samples were characterised in terms of particle size, morphology, bounds
and functional groups presence, crystallinity and thermal properties, prior to as well as upon conducted short-time micronization.

**Results and conclusions.** Successful particle size reduction to micron-scale was obtained already after 30 min of micronization in most of the samples without significant changes in thermal properties and crystallinity as well as present functional groups. An enhanced particle size decrease with prolonged micronization time (60 min) was noticed for modified sugar beet fibres with slightly wider particle size distribution compared to other examined samples. Furthermore, morphology and exposure of the present functional groups in samples were altered by the micronization which is favourable in terms of their further application as excipients in the food matrix.

**Novelty and scientific contribution.** The corresponding research reports the short-time micronization impact on sugar beet fibres and modified sugar beet fibres, inulin and sucrose for the first time hence contributing to the widening of their application as excipients in diverse products.

**Keywords**: superfine grinding; ball milling; dietary fibre; sugar beet pulp; FTIR; XRD

**INTRODUCTION**

Dietary fibres (DF) represent a vast complex group of polysaccharides, oligosaccharides, and associated compounds naturally present in plants. As a step forward to sustainability, food industry by-products (peels, pulps, cores) have emerged as DF sources with far-reaching positive effects contributing to the environment as well as human wellbeing (1,2). One of the frequently investigated by-products in this respect is the sugar beet pulp (SBP) remaining after sucrose extraction (3-6). SBP is comprised of soluble DF (pectin) as well as insoluble DF (hemicelluloses, cellulose, and lignin) accounting for total DF content in the range 74.0–84.4% (7). Additionally, well balanced soluble/insoluble DF ratio, low phytate level as well as exceptional hydration properties favour SBP application in the food industry (8,9). Nevertheless, in order to utilize the full potential of the present DF, they need to be in assimilable form and as accessible as possible. Accordingly, particle size diminishing by various mechanical treatments could be employed among which micronization or superfine grinding gain widespread use due to simple handling, maintenance, and absence of the detrimental effect on the environment (10-12). The benefits originating from micronization are multiple, from tailoring specific DF physicochemical properties (10) and increasing the extent of DF physiological function (13) to better incorporation within the food matrix and homogenisation with other present ingredients. Additionally, DF role as an excipient in emulsion stabilization and targeted delivery as well as enhancement in dissolution rate primarily relies on particle size distribution. Equipment such as ball mill is frequently used for achieving the desired micronization level (6,11,14,15). The main principle of ball mill envelopes action of pressure, collision and attrition effect.
caused by induced centrifugal force \((12,16)\). The control over micronization intensity is enabled through operational parameters adjustments such as milling speed and time, ball/powder ratio, and milling material volume \((12)\). Most of the studies employing ball mill for DF micronization were conducted under milling time 4–15 h known as long-time milling \((\text{long-time micronization})\) where structural alteration and components redistribution is achieved \((6,15,17-19)\). However, fewer studies investigated milling time in the range 5–90 min regarded as short-time milling \((\text{short-time micronization})\) and the corresponding impact on DF was different depending on the nature of the starting DF rich by-product \((14,20)\). Recently, Lin et al. \((21)\) subjected SBP to short-time micronization by using harsh thermal pre-treatment and ultrasonication which reflected in softer particle structure and reduction in particle size. Previously, Huang et al. \((6)\) reported the long-time micronization effect \((5 \text{ h})\) on SBP by assessing the particle size distribution, colour difference, physical (bulk and tap density, angle of repose and slide) and hydration properties (water and oil binding capacity), thermal characteristics and crystallographic structure. Regarding the short-time micronization by ball mill, effects on SBP were not investigated especially in its chemically modified form. Accordingly, the presented study explored the effect of short-time micronization by ball mill on structural and thermal properties of chemically modified sugar beet fibre \((\text{MSBF})\), commercial sugar beet fibre \((\text{Fibrex}^\circledR 595)\), inulin and sucrose in order to compare micronization impact among structurally different DF. Apart from particle size reduction, the aim was to reveal structural and thermal changes in the corresponding fibres induced by short-time ball milling which can further predict and tailor the use of DF in food products.

MATERIALS AND METHODS

Materials

SBP from a local sugar factory \(\text{\textquotedblleftCrvenka\textquotedblright, Crvenka, Serbia}\) was subjected to alkaline hydrogen peroxide treatment according to the procedure described in Šoronja-Simović et al. \((8)\) and subsequently passed two-stage drying \((65 \, ^\circ\text{C for 90 min and 120 min at 40 } ^\circ\text{C})\) in a convective oven \((\text{Iskramer 2K, Iskra, Horjul, Slovenia})\), grinding \((\text{Thermomix}^\circledR, \text{Vorwerk, Wuppertal, Germany})\) and sieving \((\text{type SZ–1, ZBPP, Bydgoszcz, Poland})\) to obtain modified sugar beet fibres \((\text{MSBF})\). MSBF fraction with particle size \(>315 \, \mu\text{m}\) was further micronized. Samples of commercial DF, inulin \((\text{IN})\) \((\text{Beneo GR, Orafti, Tienen, Belgium})\) and sugar beet fibres \((\text{FI})\) \((\text{Fibrex}^\circledR 595, \text{Nordic Sugar AB, Malmö, Sweden})\) with particle size \(<0.125 \, \text{mm}\), as well as sucrose \((\text{SU})\) were also examined.

Ball milling treatment

Dietary fibres samples and sucrose were micronized in a planetary ball mill PM 100 \((\text{Retsch GmbH, Haan, Germany})\) equipped with ten stainless steel balls with 10 mm diameter placed in 50 mL
cylindrical jar containing 5 g of the corresponding sample. Milling speed was set to 400 rpm with varying milling times (30 and 60 min). Accordingly, samples were assigned as 0, 30 and 60 for the initial sample and samples after 30 and 60 min of micronization, respectively, accompanied by the following designation: modified sugar beet fibres (MSBF), Fibrex (FI), inulin (IN), and sucrose (SU).

**Particle size distribution**

The particle size distribution of the samples was determined by laser diffraction using Mastersizer 2000® (Malvern Instruments Ltd., Worcestershire, UK) equipped with a Scirocco 2000 dry powder dispersion unit and particle refractive index of 1.52. Description of the particle size distribution was established by mass diameters of the volume of distribution $D_{10}$, $D_{50}$ and $D_{90}$ corresponding to 10%, 50% and 90% share of smaller to larger particles in cumulative volume, respectively. The width of the obtained distributions was depicted by span. The results in three replicates were subjected to one-way analysis of variance (ANOVA) using Statistica 14.0.0.15 software (22). Duncan’s multiple range test was applied for the determination of significant differences set at $p \leq 0.05$ between the mean values and homogeneous groups.

**Scanning electron microscopy (SEM) analysis**

DF and sucrose morphology was observed by SEM using Hitachi S-4700 (Hitachi Scientific Ltd., Japan). The sample preparation consisted of pre-coating with gold by the sputtering method. Applied magnification levels were ×70 for starting samples and ×500 for micronized samples.

**Fourier transform infrared (FTIR) analysis**

The FTIR spectra were investigated using a Thermo Nicolet AVATAR FTIR instrument (Thermo-Fisher, Waltham, USA). For the investigation, pellets were prepared by co-grinding 10 mg of sample with 150 mg of potassium bromide (KBr) and compressed with 10 tons using a hydraulic press. The FTIR spectra were acquired over the range of 4000–400 cm$^{-1}$ with a resolution of 4 cm$^{-1}$ for 128 scans. The recorded spectra were reported as absorbance in the function of wavenumber.

**X-ray diffraction (XRD) analysis**

XRD analysis of the samples was conducted by X-ray diffractometer Bruker D8 Advance (Bruker AXS GmbH., Karlsruhe, Germany) at 40 kV and 40 mA with Cu Kα radiation ($\lambda=1.5406$ Å). Diffractograms were recorded in a 2θ scan range of 3–40° with a scan speed of 0.1°/min and step width of 0.01°.
Thermal analysis

Differential scanning calorimetry (DSC) measurements were conducted using DSC 3+ (Mettler Toledo GmbH, Schwerzenbach, Swiss) with associated STARE software. An accurately weighted sample (10‒15 mg) was placed in an aluminium crucible and sealed with a lid. Measurements were performed in the synthetic air (velocity 100 cm³/min) within the temperature range 25‒300°C and heating rate of 10°C/min.

RESULTS AND DISCUSSION

Determined particle size distribution

Characteristic parameters describing the particle size distribution of DF and SU after 30 and 60 min of micronization are presented in Table 1, while the particle size distribution appearance of all samples was depicted in Fig. 1.

Samples of the same origin, MSBF and FI, exhibited different particle sizes suggesting the effect of conducted micronization as well as chemical modification. For initial MSBF particles, greater particle size reduction was observed due to a stronger attrition effect during milling compared to FI. MSBF was successfully reduced to a micron scale after 60 min of milling (\(D_{50}=84.38\ \mu m\), reduction in median diameter 8.5 times, Table 1). Conversely, with the increase in milling time of FI from 30 to 60 min a significant reduction in average particle size was not detected while the overall particle size reduction was approximately 1.2 times (\(D_{50}=54.78\ \mu m\), Table 1). A possible explanation could be that MSBF were more susceptible to the effect of attrition due to changes in structure and weakened mechanical strength after lignin dissolution as a consequence of alkaline hydrogen peroxide treatment (8). A larger reduction in average particle size after short-time ball milling of grape pomace and fibre concentrate was previously reported by Bender et al. (14). Furthermore, Huang et al. (6) reported an average SBP particle size in an ultra-micro scale (24.9 µm) after 5 h of superfine grinding. Nevertheless, a more narrowed and uniform particle size distribution of FI compared to MSBF was indicated by the obtained span values. Similar span values obtained for FI suggested an even gradual attrition effect across all particles (Table 1, Figs. 1a and 1b). Lower particle size and span values are favourable for particular applications (12) of DF such as bioactive compounds excipients or emulsion stabilisers (21), aiming to enhance the possibility of homogenization with other ingredients and dispersibility within the food matrix (11).

[Please insert Table 1 here]
Reduction in size of IN particles with prolonged milling time was neglectable since $D_{50}$ values accomplished after 30 min were almost even to those obtained after 60 min micronization (Table 1). However, smaller particle sizes were noted compared to both samples originating from SBP indicating a greater milling effect probably due to different chemical compositions as well as predominantly amorphous structure of commercial IN types (23). Increased span values for IN suggest the presence of a higher amount of fine particles but also an uneven micronization effect on IN particles which results in broadening of the particle size distribution curve (Fig. 1c).

The effect of ball milling on particle size reduction was strongly pronounced for sucrose where the median diameter decreased from 319.56 to 19.34 µm after only 30 min of milling ($D_{50}$ reduction 16.5 times). Increasing milling time caused further decrement in particle size ($D_{50}$ reduction 33 times, Table 1). However, the corresponding decrease in particle size was not evenly distributed. Smaller sucrose particles were more susceptible to the attrition effect and hence were the first to be further micronized. This increased the number of very fine sucrose particles (~2 µm) which is reflected in a very wide particle size distribution as indicated by high span values (Fig. 1d, Table 1). Additionally, the observed rise in sucrose span values with increasing milling time was the most pronounced compared to other samples. It is supposed that the span value will continue to rise with further milling due to high local mechanical energy impute leading to temperature elevation and consequently melting of the outer molecule layers (24) hence increasing stickiness. The corresponding observation demonstrates that ball milling is not an appropriate method for uniform reduction in particle size of sucrose crystals and is more applicable for lignocellulosic material such as SBP. Furthermore, short-time milling applied herein proved to be effective for particle size reduction of MSBF and FI to micron-scale, which is favourable in terms of decreasing processing costs.

**Determined morphology by scanning electron microscopy (SEM)**

Reduction in particle size of the samples is also reflected in the morphology and matrix disruption as evidenced by obtained SEM micrographs (Fig. 2). Already altered lignocellulose structure of SBP after alkaline hydrogen peroxide modification (conductive tissue segments disruption related to lignin, cellulose and hemicellulose fragmentation) (6) was further modified/changed by the attrition effect during ball milling. Rounded edge particles with irregular shape and size were observed after MSBF micronization with an increasing number of small fragments as the milling time prolonged (Figs. 2a, 2b and 2c) suggesting further fracture of the rigid structure. The corresponding fragments are usually related to lignin and cellulose moieties formed as a consequence of intermolecular bonds breakage caused by milling (14). Noticeable particle size reduction was visible on MSBF and SU micrographs as demonstrated by the particle size distribution results. These observations were
confirmed by increasing the span values of the corresponding fibre (Table 1). MSBF particles' surface was slightly coarse with a number of rifts and without visible pores. Sharper edges were observed for micronized FI particles compared to MSBF with similar furrowed surfaces interspersed with small fragments of diverse shapes (Figs. 2d, 2e and 2f).

Lumps with round edges of IN particles turned to irregularly shaped shards with sharp edges prone to aggregation as observed in micrographs, especially after 60 min of milling (Figs. 2g, 2h and 2i). A longer milling time increases the temperature of the sample as well as its amorphous portion. If the temperature of glass transition is exceeded, agglomeration of inulin particles could occur due to increased stickiness (25).

Distinctive cubic crystal structure of sucrose with clear surface and perfectly defined edges was observed in sucrose micrographs before milling (SU0, Fig. 2j). Loss of properly defined edges, the step-like structured surface (24) of remaining parts of crystals with attached and free irregularly shaped fragments in varying size were detected after micronization process (SU30 and SU60, Figs. 2k and 2l). The presence of a large number of different fragments was also confirmed by higher span values compared to DF samples implying a very wide particle size distribution (Table 1, Fig. 1d). This could be attributed to faster crystal destruction due to lower sucrose rigidity compared to DF and hence enhanced manifestation of attrition effect during milling.

**Determined functional groups and bonds by Fourier transform infrared (FTIR) analysis**

The basic structure of DF and SU in the solid state was assessed through FTIR analysis. Differences among studied carbohydrates as well as the influence of micronization were determined by the identification of obtained band patterns presented in Fig. 3. Band assignments to the corresponding functional group or bound are summarized in Table 2. Three main regions were observed on the spectra regardless of sample, O–H stretching, C–H stretching, and the fingerprint region. The most informative and hence reliable for distinction between carbohydrates, including ones comprised of glucosyl units, is the fingerprint region (26,27). According to the spectra, the general spectral profile remained unchanged in all samples regardless of applied micronization time, suggesting that the main samples’ structure was retained. Nevertheless, a decrease in particle size led to variations in bands’ intensity (absorbance) (Figs. 3a, 3b, 3c and 3d), and sporadic shifts in bands’ position (wavenumber) (FI, Fig. 3b). With an increase in milling time, bands intensity increased for DF (Fig. 3a,3b and 3c) while the opposite effect was observed for SU (Fig. 3d). Broad bands in the range 3600–3000 cm⁻¹ centred at approximately 3370–3330 cm⁻¹ depending on sample, depict O–H stretching vibrations of present OH groups within glucosyl units of sucrose and polysaccharides (27,28). A shift of the centred band in the corresponding region toward higher wavenumbers was
noted for FI after 60 min of micronization (Fl60, Fig. 3b). Sharp isolated band at ~3555 cm$^{-1}$ was noted in SU spectra which corresponds to O – H stretching in fructosyl unit (27, 29) and its intensity increased with micronization time. Furthermore, prolonged micronization induced the rise in intensity of two bands in the 3600–3000 region of sucrose. Bands observed in the corresponding region reflect vibrations of OH groups due to variations in intra- and inter-molecular hydrogen bonds, O • • • O distances and O – H • • • O angles (30). The observed wavenumber shifts were attributed to the weakening or disruption of hydrogen bonds induced by the applied mechanical force during micronization accompanied by a rise in sample temperature (14-16,31).

Bands in the range 3000–2800 cm$^{-1}$ designated the C – H stretching vibrations present in examined DF as well as SU (Table 2). C – H stretching of methyl and methylene groups within the cellulose, hemicellulose, and pectin hydrocarbon chains as SBP constituents were detected in MSBF and FI at 2930–2925 cm$^{-1}$ and 2910–2900 cm$^{-1}$, respectively (32-34). Asymmetric and symmetric C – H stretching of methyl and methylene groups within inulin structure were noticed at 2935–2930 cm$^{-1}$ and 2880 cm$^{-1}$, respectively (35,36). Characteristic stretching of the C – H bond in the sucrose glucosyl unit was detected at 2980–2975 cm$^{-1}$ (27) alongside symmetric C – H stretching of methylene groups at 2940 cm$^{-1}$ and 2920 cm$^{-1}$ (27,29). Stronger intensity of the corresponding bands regardless of the sample was observed after micronization suggesting an increased exposure and accessibility to the present functional groups of saccharides as previously observed for olive pomace and soybean residue (15,19).

Samples diversity was further depicted within the fingerprint region. The main differences observed between MSBF and FI were regarding bands indicating the presence of pectin and lignin (Table 2). Corresponding bands at ~1740, ~1510, ~1249 cm$^{-1}$ addressing C = O stretching, C = C stretching and C – O stretching within the lignin structure, respectively, were noticed only in FI and their intensity increased after micronization. C = O stretching at ~1740 cm$^{-1}$ is also an indicator of the esterified carboxyl groups' presence in SBP constituent pectin (37). The absence of the corresponding bands in the MSBF spectra could be associated with fragmentation of lignin as well as potential disruption of ester bonds among lignin and polysaccharides as a consequence of conducted alkaline hydrogen peroxide treatment (8,38).

SBP originating samples (MSBF and FI, Figs. 3a and 3b) also exhibited overlapping bands of amide I and water in the range 1700–1600 cm$^{-1}$ confirming the presence of certain proteinaceous moiety in the pectin structure (28) which was not affected by micronization. Weaker bands detected in the range 1465–1230 cm$^{-1}$ regardless of sample were associated with various C – H bending vibrations predominantly in methylene groups of monosaccharide units (SU) and hydrocarbon chains (DF) (Table 2). The strongest absorption bands for MSBF, FI and SU at ~1053 cm$^{-1}$ were even more
pronounced after micronization and ascribed to C – O stretching in carbohydrates \((39)\). Additionally, C – O – H and C –O – C stretching associated with wavenumber \(1029 \text{ cm}^{-1}\) were present in all DF samples and the strongest displayed band was detected in inulin \((26)\). Stretching vibrations of C – O, C – O – C, and C – O – H of the cyclic ring in all samples were observed in the range of \(988–879 \text{ cm}^{-1}\) \((\text{Table 2})\). Below \(858 \text{ cm}^{-1}\) various stretching and bending vibrations of glucosyl and fructosyl unit bonds were detected in the SU spectrum \((\text{Table 2})\). Furthermore, a decrease in bands’ intensity of SU after 60 min micronization was noticeable \((\text{SU60, Fig. 3d})\). Similarly, Zhao \textit{et al.} \((40)\) observed a common reduction in terms of bands intensity for ginger powder with particle size decrease. As an outcome of the applied mechanical force during grinding breakage of the intramolecular hydrogen bonds in the amorphous region of cellulose and hemicellulose occurs \((14,16)\) inducing also increased exposure of functional groups \((15)\). This reflects in variations of absorbance and wavenumber on the spectra. However, the impact of short-time micronization on main functional groups in samples was not detected since they remained unchanged.

\[\text{Please insert Table 2 here}\]

\[\text{Determined structure by X-ray diffraction (XRD) analysis}\]

\begin{itemize}
  \item X-ray diffractograms of DF and SU subjected to different milling times are shown in \textbf{Fig. 4}.
  \item Visually similar XRD patterns were obtained for MSBF and FI suggesting a semicrystalline structure with a prominent peak around \(22° (2\theta)\) and two lower peaks at approximately \(14.5–15° (2\theta)\) and \(34° (2\theta)\) as characteristic of cellulose I \((\text{Figs. 4a and 4b})\) \((44)\). Compared to FI, sharper diffraction peaks were noticed for MSBF as a consequence of conducted alkaline hydrogen peroxide treatment. Namely, partial removal of hemicellulose and lignin likewise cellulose amorphous regions hydrolysis, suggests a higher degree of crystallinity \((43)\). Nevertheless, conducted micronization induced to a certain extent an increase in the MSBF and FI diffraction peaks intensity, and sharpness but also peaks’ widening \((\text{Figs. 4a and 4b})\). Accordingly, it is assumed that applied mechanical force during micronization primarily affects the amorphous regions of corresponding samples \((15,16)\) as evidenced by FTIR analysis. However, potential changes in the crystalline structure of SBP after 5 h of superfine grinding were also reported by Huang \textit{et al.} \((6)\). For IN, a broad diffraction peak or broad halo pattern in the range \(6–25° (2\theta)\) is noticed and represents characteristic of an amorphous sample \((45)\) (IN, \textbf{Fig. 4c}). With prolonged micronization time (60 min) the corresponding halo pattern became more flattened and peak’s broadness increased suggesting the presence of a more diverse distance between present atoms due to applied mechanical force. Visually similar halo patterns of amorphous inulin with 0.9 and 15.7 g water/100 g dry inulin were reported by Ronkart \textit{et al.} \((45)\). Conversely, the extremely sharp peaks appearing in SU diffractograms were definite conformation of a pure crystalline
structure. The peaks with the highest relative intensities were detected at approximately 8.5°, 17° and 25.3° (2θ) in the starting sample and further greatly diminished and/or disappeared with an increase in micronization time (SU, Fig. 4d). Accordingly, a transformation from crystalline to amorphous structure was observed in SU samples implying sucrose crystals destruction (46) as visible on SEM micrographs (Figs. 2k and 2l).

**Determined thermal characteristics by differential scanning calorimetry (DSC)**

To assess the effect of short-time micronization on the thermal behaviour of DF and SU, DSC thermograms, at a 10°C/min heating rate were depicted in Fig. 5 while corresponding thermal parameters were summarized in Table 3. Regardless of the sample, obtained thermograms suggest endothermic reaction occurrence. An increase in micronization time usually led to similar or higher peak temperatures (t𝑝) in all samples except inulin where the opposite tendency was observed (Table 3). Visually similar thermograms were obtained for MSBF and FI with major sections representing an endothermic peak in the temperature range 43.64–149.82°C primarily attributed to the free water evaporation (Figs. 5a and 5b) (47). Recorded t𝑝 for MSBF and FI before micronization was 93.10°C and 82.65°C, respectively and increased with applied micronization time as well as the enthalpy change (Table 3). Nevertheless, previously reported higher peak temperatures for SBP (125–142°C) (6,48) suggest that the applied mechanical force herein was strong enough to release the present water without exposing the present groups susceptible to changes. Accordingly, MSBF and FI could be regarded as thermostable and hence applicable as excipients in emulsions or suspensions which include elevated temperatures (49).

All IN samples, regardless of micronization time, exhibited a broad endothermic peak (Fig. 5c). For IN30 and IN60, the endothermic peak enveloped temperature ranges from 43 to 122°C (Table 3). Slight variation in the endothermic peak appearance was observed for the starting inulin sample (IN0) in terms of increased broadness and dual peak presence, hence enveloped temperature ranges of 77.41 to 145.21°C (Table 3). The corresponding peak was assigned to water evaporation from the samples and was also previously detected by Panchev et al. (50) and Ronkart et al. (51). Furthermore, regardless of sample, thermal degradation was observed in the temperature range 220–270°C as previously reported (51).

A large endothermic peak was detected in sucrose thermograms, regardless of micronization time at onset temperature (t𝑜) nearly 189°C (Table 3) followed by a smaller endothermic peak at approximately 230°C, which represents characteristic of crystalline sucrose. The first peaks were associated with sucrose crystal lattice melting, namely loss of crystalline structure due to applied heat
The second ones were attributed to sucrose decomposition due to cleavage of disaccharide bonds followed by water elimination from monosaccharides and transformation towards volatile and non-volatile aroma compounds (46,54).

Nevertheless, alteration of the DSC curve for SU60 sample was reflected through the presence of two more peaks at lower temperatures (Table 3, Fig. 5d) suggesting the existence of an amorphous structure obtained after prolonged micronization (24) which is in accordance with the particle size (Table 1) and XRD results (SU30, SU60, Fig. 4d). The exothermic peak could be associated with crystallization while the origin of the endothermic peak could be ascribed to accelerated release of water entrapped within the mother liquor occlusions in sucrose crystals induced by micronization (52,53).

CONCLUSIONS

Short-time micronization by ball mill was employed for particle size reduction of modified and commercial sugar beet fibres, inulin, and sucrose alongside monitoring the corresponding impact on structural, thermal and physical changes. Particle size reduction by micronization was the most effective for modified sugar beet fibres and sucrose where the reported decrease in median diameter was approximately 8.5 and 33 times, respectively. Nevertheless, conducted micronization reflected unfavourably on inulin and sucrose by inducing yield losses and high span values. Regardless of sample, increased exposure and accessibility of the present functional groups were noticed as a consequence of the applied mechanical force implying the intramolecular hydrogen bonds breakage. Additionally, mentioned mechanical force induced changes primarily in the amorphous regions of corresponding samples while thermostability of modified and commercial sugar beet fibres remained unaffected by the applied force. Short-time micronization by ball mill was recognised as an effective way for improving the sugar beet fibre’s bioavailability as well as enabling their application as excipients in food products. Additionally, high suitability for industrial scale-up of the process is enabled due to the cost-effectiveness and eco-friendly approach.

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CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AUTHORS’ CONTRIBUTION

M. Djordjević analysed and interpreted the results, performed statistical analysis and wrote the manuscript. R. Ambrus was involved in ball milling and conducted analysis and manuscript revision. N. Maravić, D. Šoronja-Simović and Z. Šereš delivered the idea, research goals and methodology design. S. Vidović performed critical revision. J. Petrović was involved in conducted analysis.

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REFERENCES

4. Djordjević M, Šoronja-Simović D, Nikolić I, Djordjević M., Šereš Z, Milašinović-Šeremešić M. Sugar beet and apple fibres coupled with hydroxypropylmethylcellulose as functional ingredients in

https://doi.org/10.1016/j.foodchem.2019.05.066


https://doi.org/10.1007/s12355-013-0285-y


https://doi.org/10.1016/j.lwt.2017.08.067


https://doi.org/10.1016/j.fbp.2016.09.003


https://doi.org/10.1006/fstl.1994.1033


https://doi.org/10.1111/jfpp.13917


https://doi.org/10.1155/2016/6269302


https://doi.org/10.1016/j.ifset.2016.08.005


https://doi.org/10.1038/s41575-020-00375-4

14. Bender ABB, Speroni CS, Moro KIB, Morissono FDP, dos Santos DR, da Silva LP, et al. Effects of micronization on dietary fiber composition, physicochemical properties, phenolic compounds, and
The antioxidant capacity of grape pomace and its dietary fiber concentrate. LWT – Food Sci Technol. 2020;117:108652.

https://doi.org/10.1016/j.lwt.2019.108652


https://doi.org/10.1016/j.lwt.2020.109848


https://doi.org/10.1002/jsfa.86


https://doi.org/10.1016/j.foodchem.2005.11.034


https://doi.org/10.1111/ijfs.13632


https://doi.org/10.1016/j.lwt.2020.109526


https://doi.org/10.1016/j.indcrop.2019.05.005


https://doi.org/10.1016/j.foodres.2022.111675


https://doi.org/10.1016/j.carbpol.2015.05.026


https://doi.org/10.1007/s10973-019-08179-8

https://doi.org/10.1016/j.aaos.2020.02.002

https://doi.org/10.1002/jsfa.8672

https://doi.org/10.1016/j.cej.2012.03.085

https://doi.org/10.1016/j.jclepro.2018.01.259

https://doi.org/10.1080/05704920701829043

https://doi.org/10.1111/jfpp.12442

https://doi.org/10.1016/j.scitotenv.2017.04.138


https://doi.org/10.1016/j.carbpol.2013.11.021

https://doi.org/10.1021/bm201777j
https://doi.org/10.1016/j.foodhyd.2008.06.003


https://doi.org/10.1016/j.carbpol.2019.03.054

https://doi.org/10.3390/ma13071571


https://doi.org/10.1007/s00217-011-1584-8

https://doi.org/10.1016/j.foodchem.2009.06.035

https://doi.org/10.1016/j.jfoodeng.2017.06.016

https://doi.org/10.1007/s11095-005-7626-9

Fig. 1. Particle size distribution of dietary fibres and sucrose before and after conducted short-time micronization. Designation: modified sugar beet fibres (MSBF), Fibrex 595 (FI), inulin (IN), and sucrose (SU); 0, 30 and 60 milling time in minutes.
Fig. 2. Scanning electron micrographs of the dietary fibre samples and sucrose at ×70 and ×500 magnification depicting the effect of short-time micronization. Designation: modified sugar beet fibres (MSBF), Fibrex 595 (FI), inulin (IN), and sucrose (SU); 0, 30 and 60 milling time in minutes.
Fig. 3. FTIR spectra of dietary fibres and sucrose prior and after short-time micronization.
Designation: modified sugar beet fibres (MSBF), Fibrex 595 (FI), inulin (IN), and sucrose (SU); 0, 30 and 60 milling time in minutes
Fig. 4. X-ray diffraction patterns of dietary fibres and sucrose subjected short-time micronization. Designation: modified sugar beet fibres (MSBF), Fibrex 595 (FI), inulin (IN), and sucrose (SU); 0, 30 and 60 milling time in minutes
Fig. 5. Differential scanning calorimetry (DSC) thermograms of short-time micronized dietary fibres and sucrose. Designation: modified sugar beet fibres (MSBF), Fibrex 595 (FI), inulin (IN), and sucrose (SU); 0, 30 and 60 milling time in minutes.
Table 1. Characteristic parameters describing the particle size distribution of dietary fibres samples and sucrose affected by short-time micronization

<table>
<thead>
<tr>
<th>Sample</th>
<th>Milling time [min]</th>
<th>Particle size distribution characteristic parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$D_{10}/\mu$m</td>
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<tr>
<td>MSBF</td>
<td>0</td>
<td>(440.37±3.67)$^d$</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>(29.97±1.64)$^d$</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>(8.66±0.24)$^b$</td>
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<td>Fibrex 595 (FI)</td>
<td>0</td>
<td>(10.01±0.11)$^b$</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>(6.48±0.27)$^{ab}$</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>(6.19±0.11)$^{ab}$</td>
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<tr>
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<td>0</td>
<td>(14.43±0.42)$^c$</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>(3.48±0.50)$^a$</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>(3.49±0.45)$^a$</td>
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<tr>
<td>Sucrose (SU)</td>
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<tr>
<td></td>
<td>30</td>
<td>(2.33±0.21)$^a$</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>(1.98±0.28)$^a$</td>
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</table>

Values represent mean of three replicates. Means in the columns followed by different letters are significantly different (p<0.05), according to the Duncan’s multiple range test. MSBF—modified sugar beet fibres, $D_{10}$—mass diameter of the volume of distribution at 10% cumulative volume, $D_{50}$—mass diameter of the volume of distribution at 50% cumulative volume or volume median diameter, $D_{90}$—mass diameter of the volume of distribution at 90% cumulative volume, Span—width of the particle size distribution.
Table 2. Vibrational band assignments corresponding to the FTIR spectra of dietary fibres and sucrose affected by short-time micronization.

<table>
<thead>
<tr>
<th>MSBF</th>
<th>Inulin (IN)</th>
<th>Sucrose (SU)</th>
<th>Bond vibration</th>
<th>Reference</th>
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<td>3600-3000, 3370, 3330</td>
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<td>3500-3000</td>
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<td>3325-3323</td>
<td>O–H stretching, fructosyl unit</td>
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<td>2930-2925</td>
<td>2980-2975</td>
<td>2980-2975</td>
<td>C–H stretching, glucosyl unit</td>
<td>27</td>
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<td></td>
<td>2940</td>
<td>2940</td>
<td>C–H symmetric stretching, methylene</td>
<td>27</td>
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<td></td>
<td>2920</td>
<td>2920</td>
<td>C–H symmetric stretching, methylene</td>
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<td>2880</td>
<td>2880</td>
<td>C–H symmetric stretching, methyl</td>
<td>36</td>
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<tr>
<td>1640</td>
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<td>1640</td>
<td>C = O stretching, amide I, protein, H-O-H stretching</td>
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<td>1630-1635</td>
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<td>1435-1430</td>
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<td>Functional Group</td>
<td>Attribution</td>
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<td>1150</td>
<td>C–O–C stretching</td>
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MSBF-modified sugar beet fibres
Table 3. Thermal properties of dietary fibres and sucrose subjected to short-time ball milling

<table>
<thead>
<tr>
<th>Sample</th>
<th>Milling time (min)</th>
<th>$t_o/°C$</th>
<th>$t_p/°C$</th>
<th>$t_c/°C$</th>
<th>$\Delta H/(J/g)$</th>
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<tbody>
<tr>
<td>MSBF</td>
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<td>93.10</td>
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<td>192.23</td>
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<td>192.74</td>
<td>199.90</td>
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<td>60</td>
<td>188.84</td>
<td>191.24</td>
<td>197.48</td>
<td>-140.09</td>
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<td>67.29</td>
<td>69.44</td>
<td>69.72</td>
<td>31.09</td>
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<td>188.84</td>
<td>191.24</td>
<td>197.48</td>
<td>-140.09</td>
</tr>
</tbody>
</table>

MSBF—modified sugar beet fibres, IN—inulin, SU—sucrose, $t_o$—onset temperature, $t_p$—peak temperature, $t_c$—end set or conclusion temperature, $\Delta H$—enthalpy change of transition.