Original article
Section: Food Chemistry

Fermentation of Plant Material – Effect on Sugar Content and Stability of Bioactive Compounds

Bruno A. dos Reis, Agnieszka Kosińska–Cagnazzo*, Rudolf Schmitt, Wilfried Andlauer

*Institute of Life Technologies, University of Applied Sciences and Arts Western Switzerland
(HES–SO Valais), Route du Rawyl 47, CH–1950 Sion, Switzerland*

Key words: black tea, goji berries, fermentation, carotenoids, L–theanine, polyphenols

Extraction is a method often used to obtain products rich in bioactive compounds from plant material. Most of the solvents used for the polyphenols extraction simultaneously extract also sugars, undesirable as a component of health–promoting food. Fermentation might be a simple, cheap and efficient way of sugar elimination. In our study, black tea and goji berries, both known for their health benefits, were used and alcoholic fermentation by *Saccharomyces cerevisiae* was carried out to eliminate sugars. In the course of fermentation the concentration of polyphenols, L–theanine and carotenoids was evaluated in order to verify the preservation of selected bioactive compounds. Decreases in sugar content, formation of ethanol and yeasts growth were monitored during fermentation. The fermentation of black tea decreased the sugar concentration by 84% within 6 h without decreasing total polyphenols and L–theanine contents. Goji berry fermentation yielded a sugars decrease of 87% within 24 h, without decrease in polyphenol content. However, carotenoid content was reduced by 17%. The study showed that fermentation was an effective way to decrease sugar content in plant extracts, and therefore it might be a pertinent step to concentrate bioactives.

INTRODUCTION

The consumer awareness of the beneficial effect of bioactive compounds consumption on their health has increased significantly in recent years. Each year, several studies identify the properties and applications of new health–promoting compounds. Plant products are rich in many bioactive substances that have several beneficial effects such as phenolic compounds or carotenoids [Horszwald & Andlauer, 2011; Wang *et al*., 2010; Wildman, 2006]. In order to increase the beneficial effect of bioactives, health experts and food industries bear a special attention to the enrichment of their products in those substances. One of the means to reach this purpose is elimination of other compounds such as water or sugars. The water can be easily removed by drying, a routine operation in food industry. Lowering the amount of sugars is more complicated and there are several methods available including chromatography and fermentation [Karamać *et al*., 2012; Piasecka–Jóźwiak *et al*., 2013]. The latter is used for millennia for food preservation such as milk or vegetables, and the production of fermented beverages, *i.e.* wine or beer [Dueñas *et al.*, 2012; Paul Ross *et al*., 2002; Piasecka–Jóźwiak *et al*., 2013].

In this study, two products of plant origin: black tea and goji berries, were fermented and the stability of their bioactive compounds during this process was monitored. Black

tea was in a form of tea dust, a residue from the decaffeination process of black tea, which is currently considered as a waste. This residue holds great promise for revalorization. In fact, it contains the same concentration of bioactive compounds, L–theanine and polyphenols, as the corresponding commercial tea [Chander *et al*., 2005]. L–theanine is the dominant amino acid in tea which exerts health benefits such as improved learning and relaxing, cancer and cardiovascular diseases prevention and strengthening of the immune system [Juneja *et al*., 1999; Vuong *et al*., 2011]. Polyphenols are powerful antioxidants and prevent various diseases such as obesity, diabetes and neurodegenerative disorders as well as cancer and cardiovascular diseases [Chacko *et al*., 2010; Khan & Mukhtar, 2007].

Goji berries are small red–orange fruits from *Lycium barbarum.* They have been used for several centuries in the traditional Chinese medicine due to their anti–aging, immunity enhancing and liver protecting properties [Potterat, 2010]. Goji berries are known for their high content of polyphenols, carotenoids, amino acids, bioactive polysaccharides, vitamin C, and unsaturated fatty acids. Carotenoids are lipid–soluble pigment compounds in fruits. They are also antioxidants and several studies showed their benefit in preventing cataract, cancer, and cardiovascular diseases [Amagase & Farnsworth, 2011; Fraser & Bramley, 2004].

The aim of this study was to verify the effectiveness of fermentation as a method to reduce the sugar content without decreasing the concentration of bioactive substances in two plant products. The fermentations were performed

* Corresponding Author: Tel: +41 27 606 86 25;
E–mail: agnieszka.kosinska@hevs.ch (A. Kosińska–Cagnazzo)

by the yeast *S. cerevisiae*, and the levels of sugars, ethanol, selected bioactive compounds and yeasts growth were monitored in the course of the fermentations.

MATERIAL AND METHODS

Materials

All solvents, standards, and reagents were of analytical grade or higher. 2–propanol, gallic acid, sulfuric acid, D–(+)–galactose, D–(+)–maltose, D–(+)–mannose, D–xylose, Folin–Ciocalteu's phenol reagent, formic acid, L–(+)–arabinose, L–theanine, sucrose (β–D–fructofuranosyl–α–D–glucopyranoside) and β–carotene were purchased from Sigma–Aldrich (Buchs, Switzerland). D–(–)–fructose, D–(+)–glucose, D–galacturonic acid and ethanol were purchased from Merck (Zug, Switzerland). Dichloromethane, L–(+)–rhamnose, phosphoric acid, sodium carbonate and extra pure sodium chloride were purchased from Fisher Scientific (Reinach, Switzerland).

Chloramphenicol glucose yeast extract agar (YGC), plate count agar (PCA) and tryptic digest of casein (peptone) were purchased from Biolife (Milan, Italy). Yeast extract were purchased from Organotechnie S.A.S. (La Courneuve, France). D–(+)–glucose used in YGC broth was purchased from Sigma (Buchs, Switzerland). Deionized water used in chemical analysis was obtained using Milli–Q purification system (Millipore AG, Zug, Switzerland).

Plant material

Black tea dust was provided by Infré SA (Semsales, Switzerland). The fruits of six varieties (*Tibet, Lhasa, Nima, Red Life, Big Lifeberry, Sweet Lifeberry*) of goji berries were provided by the Agroscope Research Centre, Conthey, Switzerland. Goji berries were mixed and dried at 60°C.

Yeast preparation

Dried *Saccharomyces cerevisiae CEPPO 20,* isolated from wine fermentation, were purchased from Littorale Oenologie (Servian, France). Stock culture of *S. cerevisiae* was grown in shake flasks in 40 mL YGC broth containing 10 g/L glucose, 5 g/L peptone, 5 g/L yeast extract. The pH of the medium was set to 5.0 with 2 mol/L H_3PO_4 prior to sterilization. The cultivation was performed at 30°C in an orbital shaker (Infors–HT, Bottmingen, Switzerland) at 180 rpm for 23 h. A subculture of yeasts was prepared on YGC agar and incubated for 4 d at 25°C. This petri dish was used to prepare the fermentation inoculum.

Fermentation of black tea

Black tea dust (20.0 g) was introduced into a beaker with 380 mL of demineralized water at 80°C. After an infusion of 2 min, the content was transferred to a bioreactor Sixfors (Infors–HT, Bottmingen, Switzerland) and cooled to 30°C. *S. cerevisiae* isolated previously on YGC agar were cultured at 30°C overnight in 40 mL YGC broth in 150 mL shake flask in an orbital shaker at 180 rpm. The concentration of the inoculum was adjusted to 10^7 cells/mL and 40.0 mL were added to the bioreactor when its temperature reached 30°C. The concentration of cells was measured at 600 nm (OD600) with a Libra S12 (Biochrom, Berlin, Germany).

The fermentations were conducted at 30°C with agitation at 300 rpm. Temperature, pH and agitation speed were controlled and monitored using the software Iris v5.3 (Infors–HT, Bottmingen, Switzerland). Approximately 5–mL–samples were collected in a 15 mL polypropylene tube (VWR International AG, Zurich, Switzerland) at regular intervals in the course of fermentation with sterile pipettes (VWR International AG, Zurich, Switzerland). The growth of yeasts was followed by pour plate method with YGC and PCA agars. Four mL of the sample were passed through a 0.45 μm filter into 1.7 mL–tubes (Axygen Scientific, Union City, USA) and stored at 4°C. The latter was used for the analysis of sugars, L–theanine, ethanol and TPC.

Fermentation of dried goji berries

Dried goji berries (20.0 g) were introduced into a 250 mL beaker with 110 mL of demineralized water at room temperature and were ground in a Büchi Mixer B–400 (Büchi, Flawil, Schweiz) at 9000 rpm three times for 10 s. Then the mixture was added to the bioreactor with 270 mL of demineralized water. The addition of the inoculum, the fermentation parameters and the sampling were as described above for black tea. The samples were collected at regular intervals and sugars, ethanol, total phenolic compound (TPC) and carotenoids contents were analysed. The growth of yeasts was monitored by pour plate method with YGC and PCA agars.

Enumeration of mesophilic microorganisms

Serial 10–fold dilutions were made from the homogenized sample until the desired concentration was obtained. 1.0 mL was pipetted into the petri dishes with PCA medium in duplicate. The prepared dishes were incubated at 30°C for 72 h. Following incubation, all colonies on dishes containing colonies were counted. Negative controls were made with sterile physiological diluent. The analyses were performed in duplicate and the results were expressed as the logarithm of colony forming unit (CFU) per mL of fermentation medium.

Enumeration of yeasts

The preparation of dilutions, petri dishes and colony count were as described above for the mesophilic microorganism enumeration. The PCA medium was replaced by YGC and the incubation parameters were 25°C for 4 d. Negative controls were made with sterile diluent. The analyses were performed in duplicate and the results were expressed as the logarithm of CFU per mL of fermentation medium.

Determination of total phenolic compounds (TPC) content

TPC content was determined by Folin–Ciocalteu colorimetric method. The assay was performed as described by Horszwald & Andlauer [2011]. 25 μL of appropriately diluted samples, blanks or standards were placed into wells of a 96–wells microplate (NUNC™, NUNC A/S, Roskilde, Denmark). The analysis was executed using an Infinite M200 Pro multimode microplate reader (Tecan GmbH, Grödig, Austria) equipped with a two–channel injector system. 240 μL of Folin–Ciocalteu phenol reagent (previously 15–fold diluted with water, v/v) was added by the injector. The mixture was shaken

for 10 s and incubated in the dark for 10 min at ambient temperature. Then, 25 μL of 5% sodium carbonate was added to each well. After shaking for 10 s the mixture was again incubated in the dark for 20 min at ambient temperature. Thereafter, the microplate was automatically shaken and the absorbance was measured at $\lambda=755$ nm. Results were expressed as mg gallic acid equivalent (GAE) per g of dried plant. A blank (deionized water) and calibration solutions (gallic acid in the concentration range of 0.05 g/L to 0.5 g/L) were included in each run. Each sample from fermentation monitoring was analysed with two different dilutions in triplicate.

Determination of L–theanine content

An Agilent 1200 series liquid chromatograph (Agilent Technologies, CA, USA) comprised of an auto–sampler, a quaternary pump and a UV–DAD detector (UV Agilent Technologies 110 Series, Agilent Technologies, CA, USA) was employed. The samples were filtered through 0.45 μm filter and then transferred into 1.5 mL HPLC vials. 20 μL of each sample was injected onto a C18 column (Luna C18, 250 $\times$ 4.6 mm i.d., particle size 5 μm, Phenomenex, Torrance, CA, USA) with a security C18 guard column (4 $\times$ 2.0 mm i.d., 5 μm, Phenomenex). The mobile phase was delivered at a constant flow rate of 0.7 mL/min in a gradient mode. Solvent A was methanol containing 0.1% of formic acid and solvent B was 0.1% formic acid. The solvent A was kept at 0% from 0 to 8 min and increased to 10% from 8 to, kept at 10% from 9 to 13 min, and subsequently increased to 100% from 13 to 14 min, and kept at 100% up to 20 min. The compounds were monitored at 250 and 210 nm and the quantification was made at 210 nm. For quantification, standard solutions of L–theanine were used. Results were expressed as mg of L–theanine per g of black tea dust.

Determination of sugar content

An Agilent 1200 series liquid chromatograph (Agilent Technologies, CA, USA) comprised of an autoinjector, a quaternary pump and a refractive index detector (Agilent G1362 A RI Detector, Agilent Technologies, CA, USA) was employed. The samples were filtered (0.45 μm) and transferred into 1.5 mL HPLC vials. 20 μL of the sample were injected onto an amino column (Aminex HPX–87H Ion exclusion, 300 $\times$ 7.8 mm i.d., particle size 5 μm, Bio–Rad, Hercules, CA, USA) equipped with a precolumn at a constant flow rate of 0.6 mL/min. The mobile phase was composed of deionized water with 5 mmol/L of sulfuric acid and was delivered in an isocratic mode. The column temperature was set to 35°C. Standard solutions of different sugars were injected for identification (arabinose, rhamnose, fructose, galactose, xylose, mannose, glucose, galacturonic acid, lactose, maltose, sucrose). Calibration curves for sucrose, fructose, and glucose were constructed. Calibration was checked before every sequence by injecting a solution with 1 g/L of glucose, fructose, and sucrose. Results were expressed as g of sugar (sucrose, fructose or glucose) per L of fermentation medium.

Determination of ethanol content

The ethanol produced during fermentation of black tea was determined by gas chromatography. An Agilent 6890 series gas chromatograph (Agilent Technologies, CA, USA) comprised of an autoinjector and a flame ionization detector was employed. 400–μL samples were filtered (0.45 μm), transferred into 1.5 mL vials and 400 μL of 2–propanol solution (0.60 g/L in water) was added as an internal standard. Then, 1 μL of the sample was injected in split mode (1:10) and separated on a Varian CP7351 CP–Porabond Q Fused Silica column (Agilent Technologies, CA, USA). The mobile phase was helium delivered at a constant flow rate of 1.0 mL/min. The oven temperature was set to 40°C for 1 min, followed by a ramp of 10°C/min up to 280°C and finally this temperature was hold for 2 min. Standard solutions of ethanol with internal standard (2–propanol) were injected for calibration. Results were expressed as g ethanol per L of fermentation medium.

The ethanol produced during fermentation of goji berries was determined by HPLC along with the determination of sugars. Results were expressed as g ethanol per L of fermentation medium.

Determination of total carotenoid content

The fermented goji berry preparation (1.0 mL) was extracted three times with 5.0 mL of dichloromethane in a separating funnel. After agitating manually for 2 min the solvent was separated and subsequently evaporated to dryness under vacuum (Laborota 4011–digital, Heidolph, Schwabach, Germany) at 30°C. After the evaporation, the extract was transferred into a 10 mL volumetric flask and the volume was adjusted with hexane. Total carotenoid content was determined with a spectrophotometer (Jenway 7315, Bibby Scientific, Staffordshire, UK) at a wavelength of 450 nm in quartz cuvettes. Hexane was used as a blank and calibration was carried out with β–carotene as a standard compound in the concentration range of 0.50 mg/L to 10.0 mg/L. All measurements were performed in triplicate. The results obtained were expressed as mg of β–carotene eq. per g of dried goji berries.

Statistical analyses

The fermentations of black tea and goji berries were carried out in triplicate. Statistically significant differences ($p<0.05$) between samples were analysed using Tukey HSD method (Honest Significant Difference) and were performed using the Mintab® v.16.2.3 software.

RESULTS AND DISCUSSION

Fermentation of black tea

HPLC analysis of black tea extract confirmed the presence of glucose, fructose and sucrose. These observations are in line with the literature data [Shanmugavelan *et al.*, 2013]. All three sugars undergo fermentation with *Saccharomyces cerevisiae* [Yoon *et al.*, 2003]. Figure 1 depicts the chromatographic separation of sugars at different stages of fermentation. It shows clearly the decrease in sugar content in the course of fermentation. The fermentation of black tea was carried out after inoculation with *S. cerevisiae* in the exponential growth phase. The pH monitored during fermentation oscillated around pH 4.7. This value was near the optimal value of *S. cerevisiae* growth (pH 4.5). It assures preventing
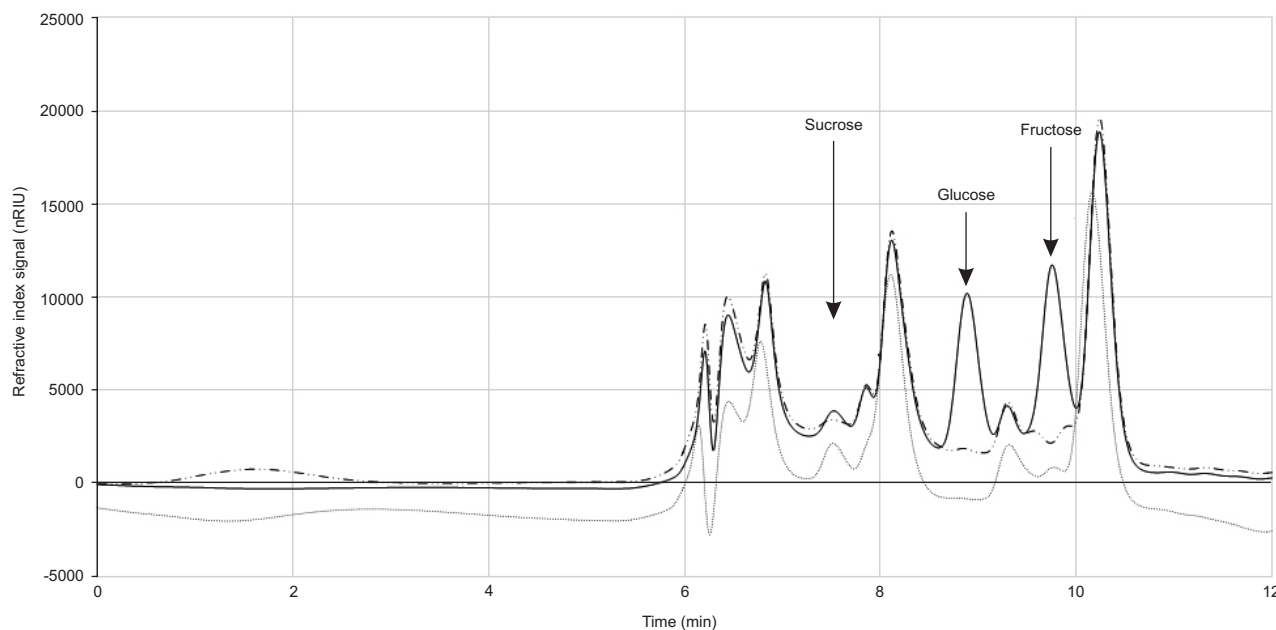
FIGURE 1. HPLC chromatogram of sugars separation of black tea after fermentation for 0 h (—), 6 h (●●●●), 24 h (– ● ● –).

or slowing down the growth or sporulation of undesired bacteria. The lack of the latter was confirmed by the analysis carried out on a non–selective medium (PCA) and on a selective medium for yeasts (YGC), which revealed only the presence of *S. cerevisiae* colonies (results not shown).

Figure 2 shows relatively fast degradation of glucose in tea fermentation broth, followed by degradation of fructose. Sucrose was present at low concentrations in black tea (0.11 g/L). Its degradation was much slower and started only after 5 h, when glucose and fructose were nearly completely degraded. Alcohol concentration reached a mean concentration of about 0.45 g/L of fermentation broth and underwent slight metabolism in the second part of the fermentation. The high variability of the sugars and ethanol concentration in three parallel fermentations results from high complexity of fermentation process and diversified batch of inoculums. Nevertheless, the effectiveness of the fermentation in decreasing sugar concentration in black tea was evident. After 5 h, glucose content decreased from 0.3 g/L to less than 0.02 g/L while fructose from 0.3 g/L to under 0.05 g/L (Figure 2).

The highest ethanol content was reached between 2 and 4 h of fermentation (about 0.4 g/L). As expected, its increase was related to sugar consumption by *S. cerevisiae*. The decrease in ethanol content after 4 h and the growth of the yeast until 24 h suggest that ethanol was consumed as a carbon source because there was no more fermentable sugar available. The low concentration of sugars and the consumption of ethanol between 5 and 24 h indicate the end of the sugar fermentation.

The stability of bioactive compounds of black tea during yeast fermentation was monitored by analysing L–theanine and polyphenol content. TPC content did not change significantly during 1 d of fermentation (Table 1). TPC content in black tea was 36.83 mg GAE/g and after fermentation it amounted to 37.29 mg GAE/g. Other studies reported values for black tea between 80 and 135 mg GAE/g [Engelhardt

et al., 2000; Khokhar & Magnusdottir, 2002]. The lower initial value of TPC content of black tea in comparison to literature data might be explained by the fact that in fermentation medium, yeasts may adsorb phenolic compounds on their apolar membrane thereby reducing TPC in solution [Sun *et al.*, 2011]. This might explain the somewhat lower values at starting time of black tea fermentation in our study. The concentration of L–theanine, another interesting bioactive substance in tea, before and after fermentation process is denoted in Table 1. The average amount of theanine in black tea before fermentation amounted to 7.6 mg/g and literature values on tea range from 1.6 to over 18 mg/g [Keenan *et al.*, 2011; Zhao *et al.*, 2012]. Interestingly, theanine concentration has not been influenced by the fermentation process.

The fermentation is known to increase the content of bioactive compound such as phenolic acids. This enrichment results from the microbial hydrolysis reaction and the breakdown of plant cells walls, which leads to the liberation or syn-
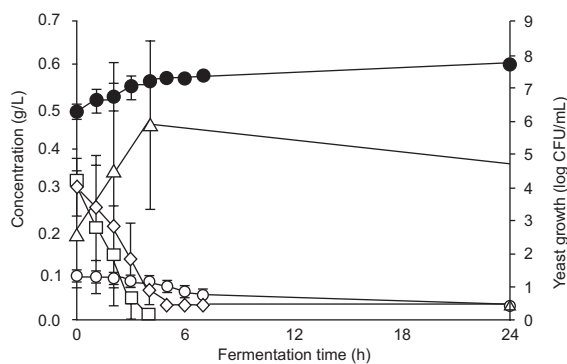

FIGURE 2. Fermentation of black tea: Concentration (mean ± SD, n=3) of glucose (□), fructose (◊), sucrose (○) and ethanol (Δ) in g/L and yeast growth (●) in log CFU/mL.

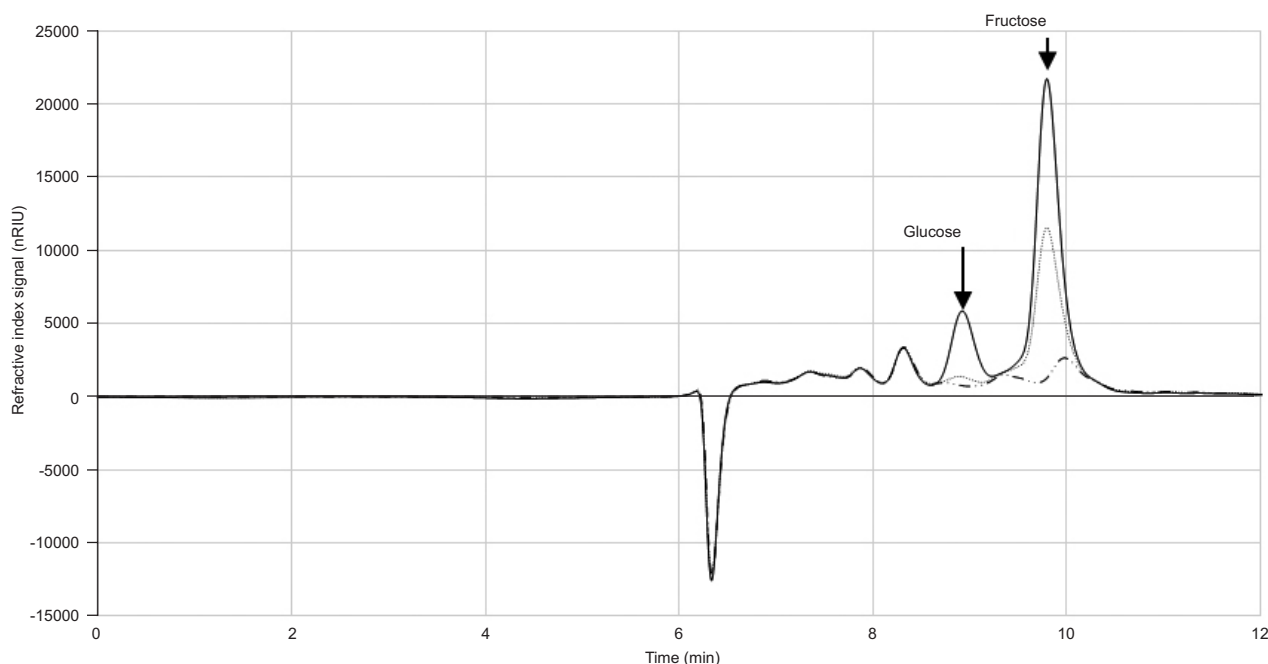FIGURE 3. HPLC chromatogram of sugars separation of goji berries after fermentation for 0 h (——), 6 h (●●●●), 24 h (– ● ● –).

TABLE 1. Total phenolic compound content, L–theanine and carotenoids content before (0 h) and after fermentation of 24 h of black tea dust and goji berries (mean ± SD, n=3). Means within the pairs (0 h and 24 h) with different letters are significantly different (Tukey's HSD, p<0.05).

	Total phenolic compounds (TPC)[1]		L–theanine[2]		Carotenoids[3]	
	0 h	24 h	0 h	24 h	0 h	24 h
Black tea	36.83±5.27[A]	37.29±6.49[A]	7.63±0.42[A]	7.59±0.33[A]	n.d.	n.d.
Goji berries	15.67±1.23[A]	17.39±0.83[A]	n.d.	n.d.	4.28±0.13[A]	3.56±0.26[B]

[1] mg gallic acid eq./g of plant material, [2] mg/g of plant material, [3] mg β–carotene eq./g of plant material, n.d. not determined.

thesis of various antioxidant compounds [Hur *et al*., 2014]. Pasha & Reddy [2005] reported an increase in the antioxidant activity after 4–day fermentation of black tea samples by *Debaryomyces hansenii*. The authors reported also an increase in theophyllin, β–caroten and riboflavin contents.

Fermentation of goji berries

Goji berries contained glucose and fructose (Figure 3). These observations were confirmed by literature data [Ye *et al*., 2006; Zheng *et al*., 2010]. Both sugars were degraded during fermentation. The pH value measured in the course of the fermentations was below 5 and showed little variations (±0.1 after 24 h and ±0.2 after 6 d). At the end of the fermentation only *S. cerevisiae* colonies were present after incubation on a non–selective (PCA) and a selective medium for yeasts (YGC).

Figure 4 shows the changes in sugars and ethanol concentration as well as yeast growth during the goji berries fermentations. The lag phase observed during fermentation of goji berries, app. 2 h, was comparable with that observed for black tea fermentation. The lag phase is affected by different factors such as the final composition of the medium (sugar content, inhibitors) or the process conditions (pH, oxygen content) [Arroyo–López *et al*., 2009]. After 6 h, most of the glucose

and nearly half of fructose was degraded. After fermentation of 24 h fructose degradation was more complete and ethanol concentration increased considerably (from 1.8±0.1 g/L to 3.1±0.2 g/L). After prolongation of the fermentation to 6 d, fructose fermentation did not continue and ethanol concen-
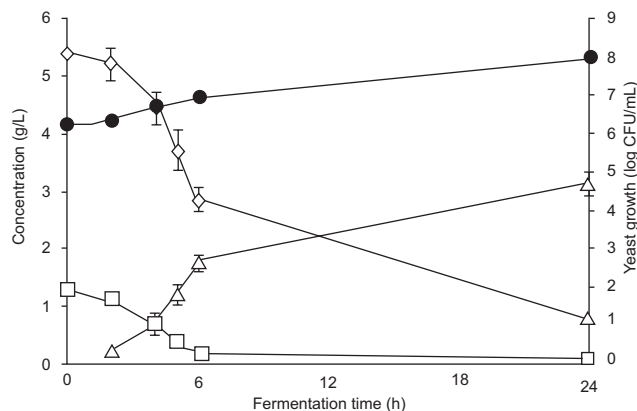

FIGURE 4. Fermentation of goji berries: Concentration (mean ± SD, n=3) of glucose (□), fructose (◊), and ethanol (Δ) in g/L and yeast growth (●) in log CFU/mL.

tration decreased. The remaining amounts of fructose might results from the presence of non–fermentable sugars showing a very close retention time to that of fructose. The correlation coefficient (R^2) between total fermented sugar (glucose and fructose) and ethanol formed was 0.99. The content of ethanol peaked after 24 h, indicating that the fermentation of sugars was completed in between 6 h and 24 h.

The phenolic compounds were not metabolized by the yeast *S. cerevisiae* (Table 1). The TPC content measured was significantly higher at the end of fermentation. This is probably due to the fact that unlike the phenolic compounds present in tea, those present in goji berries require more time to be extracted. Moreover, the ethanol formed probably helped to extract and solubilize polyphenols. TPC content measured in goji berries was 15.67 mg GAE/g before fermentation and increased to 17.39 mg GAE/g after fermentation. Another study observed similar results using Folin–Ciocalteu reagent: 10.4 mg GAE/g [Medina, 2011]. The alcoholic fermentation of mulberries did not affect the content of flavonols, however the level of anthocyanins decreased by 50% [Pérez–Gregorio *et al.*, 2011].

A second major group of bioactive substances in goji berries are the carotenoids. Their content before fermentation amounted to 4.3 mg of β–carotene/g (Table 1). The significant decrease in carotenoids content was observed. Taking into consideration oxygen–, light– and heat–sensitivity of carotenoids their loss during processing and storage is not surprising [Boon *et al.*, 2010; Rodriguez–Amaya & Kimura, 2004].

CONCLUSIONS

Tea and goji berries are two plants rich in bioactive compounds. Fermentation allows decreasing the sugar content without decreasing the level of polyphenols and L–theanine.

Black tea dust having the same composition as the corresponding commercial tea and being currently considered as waste was used as a first food model. After 6 h of fermentation, 83.5% of sugars were removed. The second food model was goji berries, considered as the new "super–fruit". In this model, a decrease of 87.3% was observed after 24 h. The sugar content did not decrease further even by extending the fermentation time. The last fermentable sugars consumed were sucrose in black tea and fructose in goji berries.

During fermentation of those two plants materials, total phenolic compounds did not decrease significantly. The concentration of the beneficial amino acid L–theanine also remained constant during fermentation of black tea. Concerning goji berries, carotenoid content decreased by 17% after 24 h.

The fermentation is an inexpensive and popular process in food and beverage industry. It can be applied in order to enrich the plant material in bioactive substances. The results of the present study might be easily transferable to other plant matrices. After the fermentation and a centrifugation or filtration step the obtained extracts can be directly spray dried if needed in powder form.

ACKNOWLEDGEMENTS

REFERENCES

1. Amagase H., Farnsworth N.R., A review of botanical characteristics, phytochemistry, clinical relevance in efficacy and safety of *Lycium barbarum* fruit (Goji). Food Res. Int., 2011, 44, 1702–1717.
2. Arroyo–López F.N., Orlic S., Querol A., Barrio E., Effects of temperature, pH and sugar concentration on the growth parameters of *Saccharomyces cerevisiae*, *S. kudriavzevii* and their interspecific hybrid. Int. J. Food Microbiol., 2009, 131, 120–127.
3. Boon C.S., McClements D.J., Weiss J., Decker E.A., Factors influencing the chemical stability of carotenoids in foods. Crit. Rev. Food Sci. Nutr., 2010, 50, 515–532.
4. Chacko S.M., Thambi P.T., Kuttan R., Nishigaki I., Beneficial effects of green tea: a literature review. Chin. Med., 2010, 5, 13.
5. Chander R., Khanna A., Raj K., Rastogi A., Antioxidant and lipid lowering activities of Indian black tea. Indian J. Clin. Biochem., 2005, 20, 153–159.
6. Dueñas M., Hernández T., Lamparski G., Estrella I., Muñoz R., Bioactive phenolic compounds of soybean (*Glycine max* cv. Merit): modifications by different microbiological fermentations. Pol. J. Food Nutr. Sci., 2012, 62, 241–250.
7. Engelhardt U.H., Lakenbrink C., Lapczynski S., Antioxidative phenolic compounds in green–black tea and other methylxanthine–containing beverages. ACS Symposium Series, 2000, 754, 111–118.
8. Fraser P.D., Bramley P.M., The biosynthesis and nutritional uses of carotenoids. Prog. Lipid Res., 2004, 43, 228–265.
9. Horszwald A., Andlauer W., Characterisation of bioactive compounds in berry juices by traditional photometric and modern microplate methods. J. Berry Res., 2011, 1, 189–199.
10. Hur S.J., Lee S.Y., Kim Y.–C., Choi I., Kim G.–B., Effect of fermentation on the antioxidant activity in plant–based foods. Food Chem., 2014, 160, 346–356.
11. Juneja L.R., Chu D.–C., Okubo T., Nagato Y., Yokogoshi H., L–theanine – a unique amino acid of green tea and its relaxation effect in humans. Trends Food Sci. Technol., 1999, 10, 199–204.
12. Karamać M., Kosinska A., Estrella I., Hernández T., Dueñas M., Antioxidant activity of phenolic compounds identified in sunflower seeds. Eur. Food Res. Technol., 2012, 235, 221–230.
13. Keenan E.K., Finnie M.D., Jones P.S., Rogers P.J., Priestley C.M., How much theanine in a cup of tea? Effects of tea type and method of preparation. Food Chem., 2011, 125, 588–594.
14. Khan N., Mukhtar H., Tea polyphenols for health promotion. Life Sci., 2007, 81, 519–533.
15. Khokhar S., Magnusdottir S., Total phenol, catechin, and caffeine contents of teas commonly consumed in the United Kingdom. J. Agric. Food Chem., 2002, 50, 565–570.
16. Medina M.B., Determination of the total phenolics in juices and superfruits by a novel chemical method. J. Funct. Foods, 2011, 3, 79–87.
17. Pasha C., Reddy G., Nutritional and medicinal improvement of black tea by yeast fermentation. Food Chem., 2005, 89, 449–453.
18. Paul Ross R., Morgan S., Hill C., Preservation and fermentation: past, present and future. Int. J. Food Microbiol., 2002, 79, 3–16.
19. Pérez–Gregorio M.R., Regueiro J., Alonso–González E., Pastrana–Castro L.M., Simal–Gándara J., Influence of alcoholic fermentation process on antioxidant activity and phenolic levels

from mulberries (*Morus nigra* L.). LWT – Food Sci. Technol., 2011, 44, 1793–1801

20. Piasecka–Jóźwiak K., Rozmierska J., Chablowska B., Stecka K.M., Skapska S., Kliszcz M., Szkudzinska–Rzeszowiak E., Starter cultures for lactic acid fermentation of sweet pepper, pattypan squash and tomatoes. Pol. J. Food Nutr. Sci., 2013, 63, 95–102.

21. Potterat O., Phytochemistry, pharmacology and safety in the perspective of traditional uses and recent popularity. Planta Med., 2010, 76, 7–19.

22. Rodriguez–Amaya D.B., Kimura M., 2004, *in*: HarvestPlus Handbook for Carotenoid Analysis International Food Policy Research Institute (IFPRI), Washington, pp. 5–7.

23. Shanmugavelan P., Kim S.Y., Kim J.B., Kim H.W., Cho S.M., Kim S.N., Kim S.Y., Cho Y.S., Kim H.R., Evaluation of sugar content and composition in commonly consumed Korean vegetables, fruits, cereals, seed plants and leaves by HPLC–ELSD. Carbohydr. Res., 2013, 380, 112–117.

24. Sun S.Y., Jiang W.G., Zhao Y.P., Evaluation of different *Saccharomyces cerevisiae* strains on the profile of volatile compounds and polyphenols in cherry wines. Food Chem., 2011, 127, 547–555.

25. Vuong Q.V., Bowyer M.C., Roach P.D., L–Theanine: properties, synthesis and isolation from tea. J. Sci. Food Agric., 2011, 91, 1931–1939.

26. Wang C.C., Chang S.C., Stephen Inbaraj B., Chen B.H., Isolation of carotenoids, flavonoids and polysaccharides from *Lycium barbarum L.* and evaluation of antioxidant activity. Food Chem., 2010, 120, 184–192.

27. Wildman R.E.C., 2006, *in*: Handbook of Nutraceuticals and Functional Foods. CRC Press LLC, Boca Raton, Florida, pp. 103–107.

28. Ye F., Yan X., Xu J., Chen H., Determination of aldoses and ketoses by GC–MS using differential derivatisation. Phytochem. Anal., 2006, 17, 379–383.

29. Yoon S.–H., Mukerjea R., Robyt J.F., Specificity of yeast (*Saccharomyces cerevisiae*) in removing carbohydrates by fermentation. Carbohydr. Res., 2003, 338, 1127–1132.

30. Zhao Y., Li P., Ge L., Wang Y., Mo T., Zeng X., Wang X., Effect of electrolyzed reduced water on chiral theanine and polyphenols in tea. Food Chem., 2012, 134, 1761–1766.

31. Zheng G.–Q., Zheng Z.–Y., Xu X., Hu Z.–H., Variation in fruit sugar composition of *Lycium barbarum L.* and *Lycium chinense Mill.* of different regions and varieties. Biochem. Syst. Ecol., 2010, 38, 275–284.