

Communication

Low-Energy Strawberry Fruits of Joly Cultivar, the First Step Towards a Novel, Food-Based Solution for the Obese Population

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Abstract: This study aimed to produce low-energy strawberries of the Joly cultivar by breeding them under different growing conditions. The efficacy of such a process was screened by the means of common physico-chemical parameters underpinned with the findings of elementary chemical analysis. The preferred strawberry breed line (treated with microbiological fertiliser of choice) showed both a decreased energy value (130 ± 6 kJ/100 g or 31.07 kcal) and an increased water content (91.0 ± 7.3 g/100 g). Due to a rich content of phenolic compounds (Total Phenolic Content /TPC/ 4184 ± 39 mg GAE/kg; Total Anthocyanin Content /TAC/ 394 ± 4 mg C3GE/kg), it also exhibited potent antioxidant activity (0.110 ± 0.008 %/ μ L, HPMC /Hydroxo-Perhydroxyl Mercury(II) Complex/) assay; $EC_{50} = 2.01 \pm 0.10$ μ g and $ARP = 0.50 \pm 0.05$ μ g⁻¹, DPPH assay). Finally, the polyphenolic extract of the same breed line showed high anti- α glucosidase activity ($IC_{50} = 19 \pm 1$ μ g/mL), being more than four-fold active compared to acarbose ($IC_{50} = 78 \pm 4$ μ g/mL), the anti-diabetic drug used as a positive control. Taken all together, the utilisation of microbiological fertiliser (*Bacillus* sp.) in tunnels might be recommended for the breeding of low-energy strawberries of the Joly cultivar (the starting point for developing of novel functional foods targeting the obese population) in the same or similar agricultural conditions, if further studies confirm its likely potential and credibility.

Keywords: *Fragaria* \times *ananassa* L. Duch; microbiological fertiliser (*Bacillus* sp.); low-energy foods; obesity

1. Introduction

Obesity represents an abnormal or excessive fat accumulation (the body mass index of 30 or more) that may be risky to health (diabetes mellitus, heart disease, malignant tumours, etc.). Unfortunately, it has nearly tripled in the last 40 years. The problem actually lies in energy imbalance between calories consumed and calories expended. However, obesity is preventable—the increased consumption of fruit and vegetables, inter alia, is highly recommended [1].

Without a doubt, food energy density (FED) significantly influences energy intake [2]. For example, plant oils and nuts exhibit high energy density [3]. In particular, the fat content leads to FED increasing [4–6]. Consequently, low-energy dense foods in the regular diet of obese adults could be of very great importance and significance in the search for the best possible food-based solution [7].

Soft fruits (including their products) are a very good source of bioactive compounds and micronutrients whose functional attributes are often linked to their antioxidant capacities [8–15]. Consequently, eating fruit may improve our well-being. However, its consumption has significantly declined during last years, due to modern eating habits [16–18].

This study aimed to produce low-energy strawberries of the Joly cultivar by breeding them under different growing conditions. Each individual breeding line was chemically analysed. In addition to this, the most promising strawberry breed line (the one with the lowest energy value) was also screened for its antioxidant and anti-diabetic effects. Finally, its likely potential in the regular diet of the obese population is briefly discussed, along with foreseeable obstacles.

2. Materials and Methods

2.1. Biological Material

This experiment (randomised by design) was conducted on Joly strawberry cultivars (at the plantation of Fruit Research Institute, Cacak, Republic of Serbia) in two cultivation systems (open field and low tunnels) with 20 plants in each treatment done in triplicate. The treatments of choice included three fertiliser variants, as follows: B—biofertiliser/microbiological fertiliser/(*Bacillus* sp.); B+C—biofertiliser + chemical fertiliser/mineral fertiliser; C—chemical fertiliser (Multi-KMg). In other words, strawberries were grown under different growing conditions: 1—open field trial; 2—tunnels, mineral fertiliser; 3—tunnels, microbiological fertiliser; 4—tunnels, with mineral and microbiological fertilisers; 5—open field trial, mineral fertiliser.

2.2. Moisture Content

The content was determined as previously reported [19].

2.3. Total Soluble Sugars and Carbohydrates

The total soluble sugar contents of fresh strawberries of Joly cultivar (starting from 1 g of fresh material) were extracted by ethanol (Sigma-Aldrich, Darmstadt, Germany) and afterwards determined gravimetrically [20]. The contents of carbohydrates were determined as described previously [19].

2.4. Glucose, Fructose and Sucrose

Glucose, fructose and sucrose in the same strawberry samples were determined according to the procedure of Steegmans, Illiaens and Hoebregs (2004) [21].

2.5. Total Proteins

The total protein contents of the aforementioned strawberries were determined using a routine methodology [22].

2.6. Total Dietary Fibers

This chemical parameter was calculated on the basis of the weight of the protein and ash and the weight of the residue, respectively.

2.7. Total Lipids

A modification of AOAC method 983.23 for food samples was applied [23].

2.8. Total Ash and Energy Value

Total ash and energy value per 100 g were determined by standard methods, EN 1135:2005 and combustion, respectively.

2.9. Elemental Analysis

Calcium, copper, iron, magnesium, mercury, sodium and zinc were determined by AAS; arsenic by HGAAS; cadmium, lead and manganese by GFAAS; and potassium by flame emission spectroscopy. Finally, phosphorus was determined by the spectrometric method EN 1136:1994.

2.10. The Preparation of Polyphenolic Extracts of Selected Strawberry Breeds

Fresh strawberries' material (1 g) was extracted with 5 mL of methanol (used as the organic solvent of choice; Sigma-Aldrich, Darmstadt, Germany), mechanically stirred for 20 min at room temperature and subsequently separated by centrifugation.

2.11. Total Phenolic Content (TPC) of the Strawberry Breed Selected

This content of the whole fruit sample (expressed as mg GAE/kg of fresh strawberry fruit weight; GA/Gallic Acid/, Sigma-Aldrich, Darmstadt, Germany) was determined applying a standard procedure for the Folin–Ciocalteu method [24].

2.12. Total Anthocyanin Content (TAC) of the Strawberry Breed Selected

This content of the whole fruit sample (expressed as mg C3GE/kg of fresh strawberry fruit weight; C3G /Cyanidin 3-Glucoside/, Sigma-Aldrich, Darmstadt, Germany) was estimated as previously described [25].

2.13. HPMC Assay

Total antioxidant activity (expressed as %/ μ L) was evaluated by HPMC (Hydroxo-Perhydroxyl Mercury(II) Complex) assay, as reported by Sužnjević et al. [26].

2.14. DPPH Assay

Anti-DPPH radical activity was determined by a very common and widely used spectrophotometric assay [27].

2.15. Anti- α Glucosidase Activity

This bioactivity was screened using a quite well-described research protocol from available literature [28].

2.16. Statistical Analysis

All experiments (means \pm SDs) were repeated thrice. ANOVA with post-hoc Tukey's HSD test ($p < 0.05$) including XLSTAT analysis was applied for the differences of the means among different treatments.

3. Results

Among the samples of fresh strawberries bred under different growing conditions, the sample 3 (microbiological fertiliser, tunnels) stood out both due to its favourable energy value per 100 g (130 kJ or 31.07 kcal) and beneficial K/Na ratio (≈ 150) (Table 1). Similar (positive) trends were observed for the contents of alkaline earth metals magnesium (202 ± 16 mg/kg) and calcium (332 ± 25 mg/kg), which were actually most abundant in the aforementioned sample. Additionally, the same sample was found to be enriched with water (91.0 ± 7.3 g/100 g) and dietary fibre (2.44 ± 0.23 g/100 g). On the contrary, it possessed the lowest content of total lipids (0.20 ± 0.02 g/100 g). However, all potentially toxic elements were present in the concentrations well below the critical levels for human health and well-being (Table 1). On the other hand, the aforementioned strawberry sample was found to exhibit a particularly high total antioxidant activity (0.110 ± 0.008 %/ μ L), due to the enriched content of phenolic

compounds (TPC and TAC 4184 ± 39 mg GAE/kg and 394 ± 4 mg C3GE/kg of fresh strawberry fruit weight, respectively). A similar trend was observed for anti-DPPH radical activity ($EC_{50} = 2.01 \pm 0.10$ μg ; $ARP = 0.50 \pm 0.05$ μg^{-1}). Finally, the polyphenolic extract of the same strawberry sample exhibited profound anti- α glucosidase activity at in vitro conditions ($IC_{50} = 19 \pm 1$ $\mu\text{g}/\text{mL}$), being more than four-fold active compared to acarbose ($IC_{50} = 78 \pm 4$ $\mu\text{g}/\text{mL}$), the anti-diabetic drug used as a positive control.

Table 1. Chemical compositions of fresh strawberries of the Joly cultivar, grown under different conditions 1–5: 1—open field trial; 2—tunnels, mineral fertiliser; 3—tunnels, microbiological fertiliser; 4—tunnels, with mineral and microbiological fertilisers; 5—open field trial, mineral fertiliser.

| Parameters | 1 | 2 | 3 | 4 | 5 |
|--------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| Total soluble sugars (g/100 g) | 7.20 ± 0.60 ^{ab} | 5.60 ± 0.70 ^{ab} | 6.60 ± 0.80 ^{ab} | 7.40 ± 0.80 ^a | 5.40 ± 0.80 ^b |
| Carbohydrates (g/100 g) | 7.70 ± 0.70 ^{ab} | 6.20 ± 0.90 ^{ab} | 7.30 ± 0.80 ^{ab} | 8.10 ± 0.60 ^a | 5.90 ± 0.70 ^b |
| Glucose (g/100 g) | 2.60 ± 0.28 ^a | 2.10 ± 0.31 ^a | 2.50 ± 0.28 ^a | 2.70 ± 0.30 ^a | 2.00 ± 0.28 ^a |
| Fructose (g/100 g) | 3.00 ± 0.39 ^a | 2.50 ± 0.38 ^a | 2.90 ± 0.38 ^a | 3.20 ± 0.39 ^a | 2.40 ± 0.39 ^a |
| Sucrose (g/100 g) | 1.60 ± 0.20 ^a | 1.00 ± 0.19 ^b | 1.20 ± 0.14 ^{ab} | 1.50 ± 0.16 ^a | 1.00 ± 0.13 ^b |
| Total dietary fibers (g/100 g) | 2.09 ± 0.23 ^a | 2.36 ± 0.26 ^a | 2.44 ± 0.23 ^a | 2.14 ± 0.25 ^a | 1.85 ± 0.22 ^a |
| Total lipids (g/100 g) | 0.20 ± 0.02 ^c | 0.30 ± 0.02 ^b | 0.20 ± 0.02 ^c | 0.30 ± 0.02 ^b | 0.40 ± 0.02 ^a |
| Total proteins (g/100 g) | 0.40 ± 0.03 ^b | 0.40 ± 0.02 ^b | 0.40 ± 0.02 ^b | 0.40 ± 0.02 ^b | 0.50 ± 0.03 ^a |
| Water (g/100 g) | 90.50 ± 7.20 ^a | 90.40 ± 7.20 ^a | 91.00 ± 7.30 ^a | 89.80 ± 7.30 ^a | 90.10 ± 7.30 ^a |
| Ash (g/100 g) | 0.30 ± 0.02 ^a | 0.30 ± 0.03 ^a | 0.30 ± 0.03 ^a | 0.30 ± 0.03 ^a | 0.30 ± 0.02 ^a |
| Energy value (kJ/100 g) | 142 ± 5 ^{ab} | 143 ± 4 ^{ab} | 130 ± 6 ^b | 155 ± 5 ^a | 138 ± 5 ^b |
| Potassium (mg/kg) | 1246 ± 94 ^b | 1283 ± 98 ^b | 2011 ± 126 ^a | 1243 ± 124 ^b | 1157 ± 94 ^b |
| Sodium (mg/kg) | 23.30 ± 2.60 ^a | <10 | 13.50 ± 2.80 ^b | <10 | <10 |
| Magnesium (mg/kg) | 129 ± 14 ^b | 137 ± 17 ^b | 202 ± 16 ^a | 129 ± 15 ^b | 124 ± 16 ^b |
| Calcium (mg/kg) | 204 ± 25 ^b | 243 ± 22 ^b | 332 ± 25 ^a | 205 ± 30 ^b | 252 ± 29 ^b |
| Phosphorus (mg/kg) | 31.0 ± 3.2 ^a | 27.3 ± 3.2 ^a | 29.5 ± 3.0 ^a | 33.8 ± 3.1 ^a | 32.3 ± 3.0 ^a |
| Zinc (mg/kg) | <1.00 | <1.00 | <1.00 | <1.00 | <1.00 |
| Copper (mg/kg) | <1.00 | <1.00 | <1.00 | <1.00 | <1.00 |
| Iron (mg/kg) | 2.48 ± 0.25 ^b | 2.30 ± 0.26 ^b | 3.21 ± 0.29 ^a | 2.49 ± 0.27 ^b | 2.21 ± 0.24 ^b |
| Manganese (mg/kg) | 2.02 ± 0.29 ^b | 2.60 ± 0.31 ^b | 3.35 ± 0.21 ^a | 2.31 ± 0.28 ^b | 2.10 ± 0.29 ^b |
| Lead (mg/kg) | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 |
| Cadmium (mg/kg) | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |
| Mercury (mg/kg) | <0.02 | <0.02 | <0.02 | <0.02 | <0.02 |
| Arsenic (mg/kg) | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 |

The values are represented as the means \pm SDs. Different superscripts (^{a,b,c,ab}) within the same row indicate the significant differences of the means, according to Tukey's HSD test ($p < 0.05$).

All fresh strawberry samples (observations) in the factor plane were classified by principal component analysis (PCA), based on their chemical compositions (Figure 1). The first two principal components (PC) represented 84.46% of the initial variability of the data (PC1—46.17% and PC2—38.29%). Factor loadings of PC1 and PC2 are listed in Table 2. In brief, PC1 was strongly correlated with potassium, iron, magnesium, manganese and water, unlike PC2 with glucose, fructose, sucrose, carbohydrates and total soluble sugars (factor loadings >0.8). Consequently, PC1 and PC2 can be interpreted as mineral and sugar composition components, respectively. By the means of the chemical analyses, the following clustering pattern was observed: 1st group—the strawberry samples 1 and 4; 2nd group—the strawberry samples 2 and 5; 3rd group—the strawberry sample 3. Indeed, the most promising breed line (sample 3) was totally separated from the rest of the samples examined.

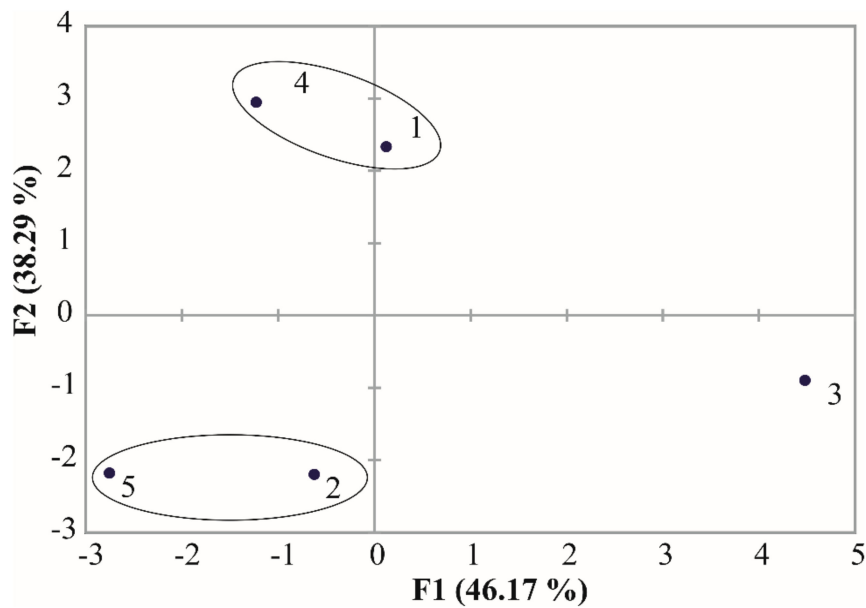


Figure 1. Principal component analysis (PCA) of Joly strawberry samples in PC1 versus PC2 plane.

Table 2. Factor loadings of the principal components (PCs) 1 and 2 obtained by principal component analysis for the chemical composition of the fresh strawberries samples examined.

| Parameters | PC1 | PC2 |
|----------------------|---------|---------|
| Total dietary fibres | 0.7865 | −0.1029 |
| Potassium | 0.9562 | −0.1682 |
| Phosphorus | −0.4186 | 0.5994 |
| Glucose | 0.4111 | 0.9052 |
| Fructose | 0.3442 | 0.9191 |
| Calcium | 0.7333 | −0.5861 |
| Sucrose | 0.1173 | 0.9734 |
| Iron | 0.9597 | 0.0912 |
| Magnesium | 0.9461 | −0.2241 |
| Manganese | 0.8588 | −0.3366 |
| Water | 0.8702 | −0.3188 |
| Sodium | 0.5468 | 0.3992 |
| Carbohydrates | 0.3480 | 0.9284 |
| Total soluble sugars | 0.3050 | 0.9511 |
| Total lipids | −0.8086 | −0.4356 |
| Energy value | −0.6016 | 0.6195 |

4. Discussion

Unlike the sample 3, industrial strawberry products (termed exhausted strawberry flesh) did exhibit much higher energy values—310 and 276 kcal/100 g of dry weight, respectively [29]. Though electrochemical assays are often applied on food and beverage samples, that is really not true for HPMC assay and fruit samples of any kind [30–34], in spite of the fact that HPMC actually represents a cheap and time-saving alternative, compared to conventional spectrophotometric and chemiluminescent hydrogen peroxide assays. Additionally, a previously screened sample of the whole strawberry of the Clery cultivar (its polyphenolic extract) did exhibit 2.62-fold lower total antioxidant activity ($0.042 \pm 0.003 \text{ %}/\mu\text{L}$, determined by the same polarographic assay). Such a finding was directly a consequence of much lower content of phenolic compounds found for the aforementioned sample of Clery cultivar (TPC and TAC $1597 \pm 15 \text{ mg GAE/kg}$ and $351 \pm 4 \text{ mg C3GE/kg}$ of fresh strawberry weight, respectively) [34]. The abundance of phenolic compounds (recorded for the sample 3) may actually

contribute a lot both to the anti-obesity and anti-hypertensive effects of this particular strawberry breed [11,35,36].

Acarbose, miglitol and voglibose are well known α -glucosidase inhibitors capable of suppressing postprandial hyperglycaemia [37–40]. Indeed, the overall effect of α -glucosidase inhibition may prevent the development of diabetes [41]. Additionally, edible blackberry water and ethanol extracts have been linked with anti-diabetic effects [42]. In addition to this, some organic extracts of raspberry fruits exhibited a potent anti- α glucosidase activity [43]. Compared to Chinese coloured grains, the polyphenolic extract of the strawberry fruit sample 3 was found to be at least 714-fold more active [44]. In terms of their side effects, natural α -glucosidase inhibitors are generally considered more promising (safer) than synthetic ones [45].

Undoubtedly, eating fruit is often linked to a number of obstacles. For example, the pieces spoil too quickly (in other words, there is a very short time where fruit is at its best quality), should be prepared (cut and peeled) before consuming, etc. There is constant interest in offering the consumers new food products of substantial quality that will stimulate its consumption [46]. Consequently, finding an easy and elegant way (or technique) for the preservation and/or storage of sensitive fruits such as strawberries does represent extremely attractive research goal. Unfortunately, traditionally used preservation techniques affect, to a considerable extent, the sensorial and nutritional properties of fruit in general, including soft fruit [47].

5. Conclusions

In summary, the use of a microbiological fertiliser in tunnels (*Bacillus* sp.) might be recommended for the breeding of low-energy strawberries of the Joly cultivar in the same or similar agricultural conditions, if further studies confirm its likely potential and credibility. Low-energy strawberries described herein should be treated as a good starting point for an innovative, food-based solution in the form of effective functional foods targeting the obese population. Indeed, the research on dried strawberry juices (obtained from low-energy strawberries of Joly cultivar, conventionally dried for 48 h at room temperature) as a reliable option for obese people is currently in progress in our labs.

Author Contributions: All authors contributed to the design of this study. M.S. and S.O. performed most of the experimental work, kindly supported by M.P. and D.M. Meanwhile, B.P., M.S. and S.O. were the key authors who analysed the obtained results; D.M. performed all statistics. Finally, B.P. wrote this manuscript, aided by M.S. who critically read it more than once, each time coming back with extremely informed thoughts.

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