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# Sustainable Development of Apple Snack Formulated with Blueberry Juice and Trehalose

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**Abstract:** Novel products that carry concrete and relevant health benefits, with texture and flavor not substantially different from already available products, are generally well accepted by consumers. Vacuum impregnation is a non-thermal technology that allows the enrichment of fruit with different ingredients in solution. The characteristic of the resulting product is a combination of both the solid matrix and the impregnation solution. This work aimed at: (i) evaluating the effect of trehalose on anthocyanin retention after drying of apple snacks vacuum impregnated with blueberry juice; (ii) modelling the air-drying kinetic, proposing an image analysis approach to monitor the drying process. Four mathematical models successfully fitted the drying experimental data, obtainingequations that could be used in the implementation of this process at industrial scale. The drying kinetics of samples impregnated with blueberry juice and trehalose were faster when compared to the control sample. Samples impregnated with blueberry juice and 100 g/kg of trehalose retained nearly four times more anthocyanin after drying when compared to the control.

**Keywords:** vacuum impregnation; sustainable development; mathematical modelling



**Citation:** Castagnini, J.M.; Tappi, S.; Tylewicz, U.; Romani, S.; Rocculi, P.; Dalla Rosa, M. Sustainable Development of Apple Snack Formulated with Blueberry Juice and Trehalose. *Sustainability* **2021**, *13*, 9204. https://doi.org/10.3390/su13169204

Academic Editor: Alessandra Durazzo

Received: 15 July 2021
Accepted: 14 August 2021
Published: 17 August 2021



## 1. Introduction

Nowadays, consumers are increasingly aware of the nutritional value of food and its impact on health and proper functioning of the body [1]. Studies show that the commercialisation of novel food products is most successful when they are developed based on consumer orientation [2,3]. Novel products that carry concrete and relevant benefits, but with texture and flavor not substantially changed from already available products, are likely to be well accepted by consumers [4].

On the other hand, the United Nations proposed The Sustainable Development Goals (SDGs) calling all countries to promote prosperity while protecting the planet. In particular, SDG 12 is related to responsible consumption and production in the entire food system. The re-use of by-products from food processing and their re-introduction in the production cycle [5] is a strategy that is worth considering for the development of more sustainable technologies [6]. In this sense, a very interesting initiative born in the USA is called 'Upcycled food'. The Upcycled Food Association states that upcycled products prevent food waste by creating new, high-quality products out of surplus food. This is an innovative approach to prevent food waste, because being the first consumer product-based solution makes it highly scalable and economically sustainable [7]. Although many actors and industrial sectors could help to tackle the limitation of the traditional food system approach, researchers can contribute by innovations able to create a better and more nutritious diet and a sustainable food system [8].

Blueberries are very attractive for consumers, because of their flavour but also for their nutritional properties. Numerous scientific articles have stated that blueberries are an excellent source of health-related compounds [9–14]. Anthocyanins are the most prevalent family of flavonoids in blueberries [9,15,16]. Different studies have confirmed their anti-inflammatory and anti-carcinogenic properties and their cardiovascular protective effects [9]. It is worth mentioning that the antioxidant compounds present in blueberries diminish the risk of coronary diseases, as well as prevent the oxidation of cholesterol, thus lowering the risk of atherosclerosis. These compounds might also avert neurodegenerative disorders [17]. Although the fresh fruit market is the most important sector, the development and study of processes suitable for the recovery of interesting bioactive compounds is needed in particular for those fruit products that do not reach the quality parameters requested by the fresh market [18].

Vacuum impregnation (VI) is a non-thermal technology that allows the direct formulation of fruits [19,20], achievable through the selective incorporation of solutes, without modifying the food integrity. The characteristic of the resulting product is a combination of both the solid matrix and the impregnation solution.

After VI, a stabilization process to reduce the water activity, such as drying, is needed to obtain fruit products with a longer shelf-life. Hot air drying consists in the transfer of heat from the hot air to the product by convection; similarly, the evaporated water is transported to the air by convection [21,22]. However, it is well known that vitamins, colourants, and antioxidants are sensitive to high air temperatures; this imposes the use of low dehydration temperatures and drying intensity decrease [23]. On the other hand, even when drying is carried out at a low temperature, a prolonged exposition to the oxygen could substantially decrease the nutrient content of treated products [24]. Therefore, the study of the drying kinetics is of special interest to select the best process conditions. Besides, the application of mathematical modelling is important for the process implementation from the laboratory to the industrial scale.

The use of computer vision for the industrial control of processes is growing. Single-point spectroscopy and machine vision are techniques able to non-destructively assess the product quality changes during drying [25]. Different authors reported applications aimed to monitor colour, size, shape, shrinkage and moisture changes in different foods during processing [26–30].

During drying, the use of different thermo-protective molecules could help in improving the nutritional content and the sensorial properties, reducing the colour changes and modifying textural properties, and water-related properties, modifying the water content and water activity. Trehalose is an important disaccharide able to maintain and preserve a wide group of biologically active molecules [31]; it possesses a high glass transition temperature, ranging from 70 to 113 °C, which can provide good physical stability for the matrix where it is used [32].

This work aimed both at modelling the air-drying kinetic of apple snacks, proposing an image analysis approach to monitor the drying process, and at evaluating the effect of trehalose on the anthocyanin content of vacuum impregnated apple slices with blueberry juice.

## 2. Materials and Methods

### *2.1. Raw Material*

Apples (var. *Granny Smith*) purchased from a local market in Cesena (Italy) were stored at 4 ± 1 °C and 80% relative humidity in the darkness before the analyses. The apples were washed and cut into discs of 60 mm external diameter, 23 mm internal diameter and 5 mm thickness.

Undersized and overripe blueberries were crashed with a food processor (Russel Hobbs, 27700-56) to obtain blueberry juice. A depectinization process was carried out as described by Castagnini et al. [33]. The enzymatic depectinization of 100 g of blueberries was carried out for 78 min at 50 °C with 4 mg of Lafase® Clarification and 8 mg of Lafase ®

He Grand Cru. Finally, the blueberry juice was filtered by a 0.5 mm sieve and pasteurized at 77 ± 1 °C for 85 s in a water bath.

Trehalose dihydrate was purchased from ACEF S.P.A. (Piacenza, Italy).

### 2.2. Vacuum Impregnation (VI)

Blueberry juice (control) and blueberry juice with two concentrations of trehalose (Cargill, Milan, Italy), 50 and 100 g of trehalose/kg of juice, were utilized as impregnation solutions for apple samples. VI was carried out at 25 ± 1 °C in a closed chamber connected to a vacuum pump and an automatic vacuum controller system (AVCS, S·I.A., Bologna, Italy). The treatment duration was 10 min at 200 mbar (absolute pressure) and 10 min at atmospheric pressure as reported by Betoret et al. [34]. At the end of the treatment, impregnated apple samples were removed from the solutions and wiped with absorbing paper and their weight was recorded. At least three independent impregnation cycles were carried out for each sample.

### 2.3. Air-Drying

Vacuum impregnated (VI) samples were air-dried using a hot air cabinet dryer (POL-EKO-APRATURA SP.J., PL) at 50 °C for 8 h. The air velocity was 2 m/s, and the air renewal fee was 50%. The sample weight was recorded every 15 min for the first 2 h and every 30 min till the end of drying. Drying was performed until the samples reached a water activity value below 0.4.

All obtained samples with related abbreviations are reported in Table 1.

**Table 1.** Obtained vacuum impregnated apple sample and related abbreviations.

| Sample Code | Description |
|---|---|
| VI Control | Sample vacuum impregnated with blueberry juice |
| VI 50 | Sample vacuum impregnated with blueberry juice with 50 g/kg of trehalose |
| VI 100 | Sample vacuum impregnated with blueberry juice with 100 g/kg of trehalose |
| D Control | Dried VI control sample |
| D 50 | Dried VI 50 sample |
| D 100 | Dried VI 100 sample |

### 2.4. Mathematical Modelling

To study the drying process and compare the obtained different drying kinetics, data modelling was applied. In this research work, four models, listed in Table 2 were evaluated. Drying curves were plotted as a function of dimensionless moisture ratio (MR), that was calculated as follows:

$$MR = \frac{M_t - M_e}{M_0 - M_e} \quad (1)$$

where $M_e$ is the equilibrium moisture content, $M_0$ is the initial moisture content, and $M_t$ is the moisture content at each considered step of the process. All the recorded moisture content was expressed as g water/g dry matter. Equilibrium moisture was obtained when a constant weight for three consecutive measurements were reached ($\Delta w < 0.0005$ g) after the end of drying (below $a_w = 0.4$, as reported in Section 2.3).

**Table 2.** Selected mathematical equations used to model the drying kinetics.

| Model Name | Model Equation | Reference |
|---|---|---|
| Newton (Lewis) | $MR = e^{(-k.t)}$ | [35] |
| Page | $MR = e^{(-k.t^n)}$ | [35] |
| Weibull | $MR = e^{-(\frac{t}{\alpha})^{\beta}}$ | [36] |
| Fick | $MR = \frac{8}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{2n+1} e^{(-\frac{(2n+1)^2 \pi^2 D_e t}{4L^2})}$ | [37] |

Regression analysis was performed using Matlab (The MathWorks Inc., Natick, MA, USA). To evaluate the goodness of fit of every model, correlation coefficient ($R^2$), root mean square error (*RMSE*), and sum squared error (*SSE*) were calculated as follows:

$$RMSE = \sqrt{\frac{\sum_{i=1}^{N}\left(MR_{i,p} - MR_{i,e}\right)^2}{N}} \quad (2)$$

$$SSE = \frac{\sum_{i=1}^{N}\left(MR_{i,p} - MR_{i,e}\right)^2}{N} \quad (3)$$

where $MR_{i,p}$ is the predicted dimensionless moisture ratio, $MR_{i,e}$ is the experimental dimensionless moisture ratio, and $N$ is the number of observations. The higher $R^2$ values (near 1) and the lower *SSE* and *RMSE* values indicate that the model fits better the experimental data. The effective water diffusion coefficient during drying was calculated by using the simplified equation of Fick's diffusion second law for an infinite slab.

### *2.5. Analytical Determinations*

#### 2.5.1. Moisture Content and Water Activity

Moisture content (g water/g dry matter) was determined gravimetrically by drying the samples at 70 °C until a constant weight was achieved [38]. Water activity was measured at 20 °C by using Aqualab (Decagon Devices Inc., Pullman, WS, USA) $a_w$ meter. Both analyses were carried out in triplicate on randomly selected sub-samples for each sample.

#### 2.5.2. Computer Vision System (CVS)

The size and shape of each sample were measured using a Computer Vision System (CVS) consisting of an illumination source, a colour digital camera (CDC), and an image processing software. Apple samples were placed inside a dark box to exclude external light, and RGB images were acquired by a CDC (Mod. D7000, Nikon, Japan) with a 60 mm lens (Mod. AF-S Micro f/2.6G ED, Nikkor), located vertically over the sample at a distance of 35 cm and connected to a PC. The lighting system consisted of four daylight fluorescent lamps (60 cm in length) connected to an electronic ballast to ensure uniform illumination, with illuminant D65 and a colour temperature of 6500 K. The pre-processing of RGB images, segmentation and area quantification were performed with Python. The area was calculated as the number of white pixels and considering that 1 cm was equal to 188 pixels for the image acquisition configuration. The shrinkage of apple samples during drying was calculated according to the Equation (4).

$$\%\ Shrinkage = \frac{A_0 - A_t}{A_0} \cdot 100 \quad (4)$$

where $A_0$ is the initial sample area and $A_t$ is the area reached at each considered drying time.

#### 2.5.3. Total Anthocyanin Content

To determine the total anthocyanin content in solid samples, each slice was milled and a solid-liquid extraction was performed with methanol acidified with hydrochloric acid (HCl) (0.1 mL/100 mL) [39]. For the blueberry juice, the sample was centrifuged at 3000× *g* and measured directly without extraction.

Total anthocyanin content was measured by the pH differential spectrophotometric method developed by Giusti and Wrolstad [40]. Anthocyanin content was quantified using the molar extinction coefficient for Cyanidin-3-*O*-glucoside (2690 $m^2$/mol). Results were expressed as milligrams of Cyanidin-3-*O*-glucoside equivalents per gram of sample.

### 2.6. Statistical Analysis

The significant differences among samples were analysed by means of one-way ANOVA (analysis of variance, $p$-level < 0.05, post-hoc Tukey, Statgraphics Centurion XVI software).

## 3. Results and Discussion

VI is a mass transfer operation that takes place in complex cellular matrix and porous structure. The application of this technology for the development of new food products requires an impregnation medium to be introduced into the pores or intercellular spaces of the food matrix. In these research trials the apple samples weight increase, due to the impregnation process, was of 13.6 ± 3.6% ($w/w$); similar results were obtained by [8,39,41] on apple samples impregnated with microalgal suspension, blueberry juice and calcium lactate, respectively.

After impregnation, the drying process of each sample was monitored for 8 h. In Table 3, water activity and moisture content values of each VI apple sample before and after drying are presented. All dried samples showed water activity below 0.4 that guarantees the stability of the final product [42,43]. Moisture contents of both VI and dehydrated samples decreased when trehalose was added in the blueberry juice. This could be related to the solid gain as a consequence of vacuum impregnation. In fact, the water loss and solid gain was calculated as reported by Dellarosa et al. [44]. For the VI Control sample the water loss was 0.142 ± 0.001 g and the solid gain was −0.0060 ± 0.002 g; for the VI 50 and VI 100 the water loss was 0.12 ± 0.02 g and 0.115 ± 0.008 g, and the solid gain was 0.021 ± 0.003 g and 0.022 ± 0.001 g, respectively.

**Table 3.** Water activity and moisture content of all VI apple samples.

| Sample | $a_w$ | Moisture g water/g d.m. |
|---|---|---|
| VI Control | 0.981 ± 0.003 $^{a}$ | 6.7 ± 0.2 $^{a}$ |
| VI 50 | 0.983 ± 0.005 $^{a}$ | 5.5 ± 0.3 $^{b}$ |
| VI 100 | 0.969 ± 0.014 $^{a}$ | 5.5 ± 0.1 $^{b}$ |
| D Control | 0.387 ± 0.039 $^{a}$ | 0.27 ± 0.01 $^{a}$ |
| D 50 | 0.324 ± 0.025 $^{b}$ | 0.15 ± 0.08 $^{ab}$ |
| D 100 | 0.352 ± 0.034 $^{ab}$ | 0.117 ± 0.009 $^{b}$ |

Different lowercase letters in columns indicate significant differences ($p$ < 0.05) between the same samples before (VI) and after drying (D).

The anthocyanin content of the blueberry juice obtained by enzymatic extraction was 172.56 ± 6.64 mg/L. The anthocyanin contents of VI and VI dried apple sample are presented in Table 4. The presence of trehalose did not influence the content of anthocyanins in VI samples; as expected, however, after drying, samples that contained blueberry juice enriched with trehalose were characterized by a higher anthocyanin content. In particular, the samples D 50 and D 100 showed an anthocyanin retention after drying of 9.7 ± 2.2 and 27 ± 2.5% respectively, when compared to the initial anthocyanin content, while in the D Control sample it was only 7.0 ± 1.7%. The degradation of anthocyanins depends, among other factors, on polyphenol oxidase activity, organic acid content, pH and temperature [45,46]. Anthocyanins are also easily susceptible to the degradation during storage and especially during heat processing [47,48]. Besides, the degradation of phenolic compounds during drying has also been attributed to either the binding of polyphenols with other compounds, to alterations in the chemical structure [49] and to the drying duration [39,50,51]. In order to mitigate the losses of antioxidant compounds Barani et al. [52] proposed different pre-treatments with citric acid, ascorbic acid, tartaric acid and sucrose. In the case of our study, the higher retention of anthocyanins in the samples D 50 and D 100 could be attributed to the trehalose thermo-protective effect; it is indeed known that this disaccharide possesses an extraordinary ability to stabilize biomolecules during thermal treatments [53,54].

**Table 4.** Anthocyanin content (on a dry basis) of VI apple samples before and after drying.

| Sample | VI (mg/kg d.m.) | Dried (mg/kg d.m.) |
|---|---|---|
| Control | 2405 ± 45 [a] | 167 ± 43 [a] |
| 50 | 2401 ± 35 [a] | 233 ± 48 [a] |
| 100 | 2403 ± 14 [a] | 655 ± 63 [b] |

Different lowercase letters in columns indicate significant differences ($p < 0.05$) between the samples.

The drying curves of different samples are showed in Figure 1. Both samples impregnated with trehalose, VI 50 and VI 100, showed a faster drying, reducing the time needed to achieve a dimensionless humidity of 0.2 in 10% and 23%, respectively, when compared with the control sample. This effect is related to the trehalose impregnated in the samples.

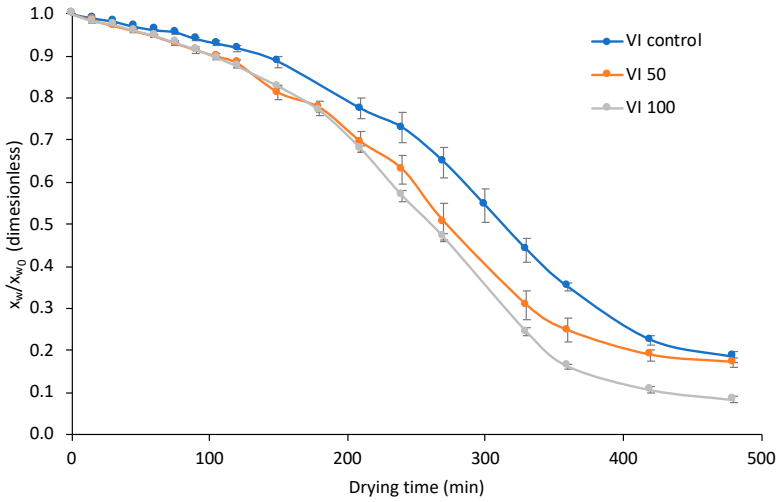


**Figure 1.** Dimensionless humidity of vacuum impregnated apple samples during drying time.

The recorded drying data were modelled with the four proposed models. The Newton model is the simplest because it considers only one kinetic constant (k). The higher the drying velocity, the higher the k constant. In the case of the Page model, it considers the kinetic constant (k) and introduces an empirical exponent (n) to overcome the shortcomings of the Newton model (also known as the exponential model) [55]. The Weibull model considers the scale parameter (α) and the shape parameter (β). The first is the kinetic constant of the model and represents the time needed to accomplish approximately 63% of the drying; while the latter is related to the velocity of the mass transfer at the beginning of the drying (the lower is β, the faster is the drying rate at the beginning). Finally, for the classical Fick's model, the effective diffusion coefficient ($D_e$) was calculated. The kinetic parameters obtained from each model are presented in Table 5. Although statistical differences were only found for the Weibull and Fick model, the higher drying rate of the samples impregnated with trehalose was confirmed by almost all the models. This higher drying rate could be related to the higher initial solid content as a consequence of vacuum impregnation, but also to the trehalose ability to form hydrogen bonds with the biomolecules that allows to stabilize cells and tissues preserving viability and structures [56]. In fact, trehalose has an unusually high destructuring effect on the hydrogen bonded network of water [57,58]. Moreover, a 'water-replacement' hypothesis proposed by Crowe et al. [59] which assumes that sugars hydrogen-bond (HB) to biomolecules during dehydration or freeze-drying, acting as substitutes of hydration water molecules [57].

**Table 5.** Parameters for each model.

| Sample | Newton | Page | | Weibull | | Fick |
|---|---|---|---|---|---|---|
| | k ($s^{-1}$) | k ($s^{-n}$) | n | $\alpha$ (s) | $\beta$ | $D_e$ ($m^2 \cdot s^{-1}$) |
| VI Control | $9.3 \pm 0.8 \times 10^{-5}$ a | $1.6 \pm 0.8 \times 10^{-5}$ a | $1.33 \pm 0.09$ a | $3.0 \pm 0.1$ a | $1.34 \pm 0.07$ a | $2.7 \pm 0.2 \times 10^{-10}$ a |
| VI 50 | $1.1 \pm 0.8 \times 10^{-4}$ a | $3 \pm 1 \times 10^{-5}$ a | $1.25 \pm 0.08$ a | $2.60 \pm 0.09$ b | $1.28 \pm 0.06$ a | $3.2 \pm 0.02 \times 10^{-8}$ b |
| VI 100 | $1.1 \pm 0.7 \times 10^{-4}$ a | $2 \pm 1 \times 10^{-5}$ a | $1.29 \pm 0.09$ a | $2.6 \pm 0.1$ b | $1.27 \pm 0.06$ a | $3.30 \pm 0.09 \times 10^{-10}$ b |

Different lowercase letters in columns indicate significant differences ($p < 0.05$) between the samples.

In Table 6 the goodness of fit of each model is reported. The R-square higher than 0.95 indicates that all models explain 95% of the variable variance. Besides, the very low SSE and RMSE values indicate that all models fit very well the experimental data.

**Table 6.** The goodness of fit for Newton, Page, Weibull, and Fick model.

| Model | R-Square | SSE | RMSE |
|---|---|---|---|
| Newton | $0.972 \pm 0.006$ | $0.15 \pm 0.04$ | $0.054 \pm 0.006$ |
| Page | $0.992 \pm 0.001$ | $0.042 \pm 0.007$ | $0.029 \pm 0.002$ |
| Weibull | $0.992 \pm 0.001$ | $0.042 \pm 0.007$ | $0.029 \pm 0.002$ |
| Fick | $0.964 \pm 0.002$ | $0.5 \pm 0.2$ | $0.12 \pm 0.02$ |

During drying, the cellular membranes of fruit and vegetables collapse because of water loss [25]. As a result of this microscopic phenomenon, a reduction in the shape and size of food tissues, commonly called shrinkage, takes place [60]. Different authors confirmed that a linear correlation exists between moisture content and shrinkage phenomenon [26,61,62].

Using a developed python script, it was possible to segment all the apple sample images and transform them into binary images to measure the area. The resulting images for the control sample are presented as an example in Figure 2.

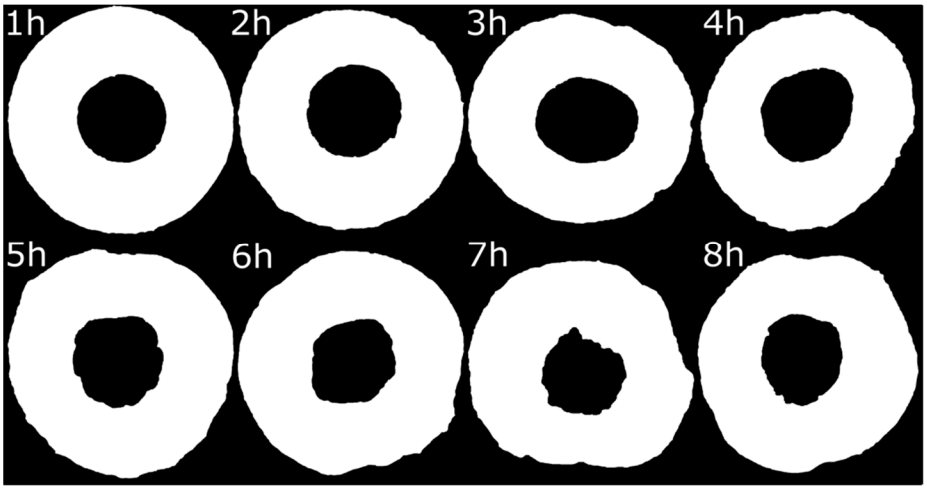


**Figure 2.** Binary images of apple samples taken during 8 h drying.

The shrinkage calculated from the data extracted from the binary images of each sample was plotted versus the drying time in Figure 3. Data was fitted with a second order polynomial model with R-square of 0.996, 0.973 and 0.979 for VI Control, VI 50 and VI 100, respectively. It could be observed that for the samples impregnated with trehalose the shrinkage phenomena diminishes, which could be related to the structuring effect of trehalose [31,32].

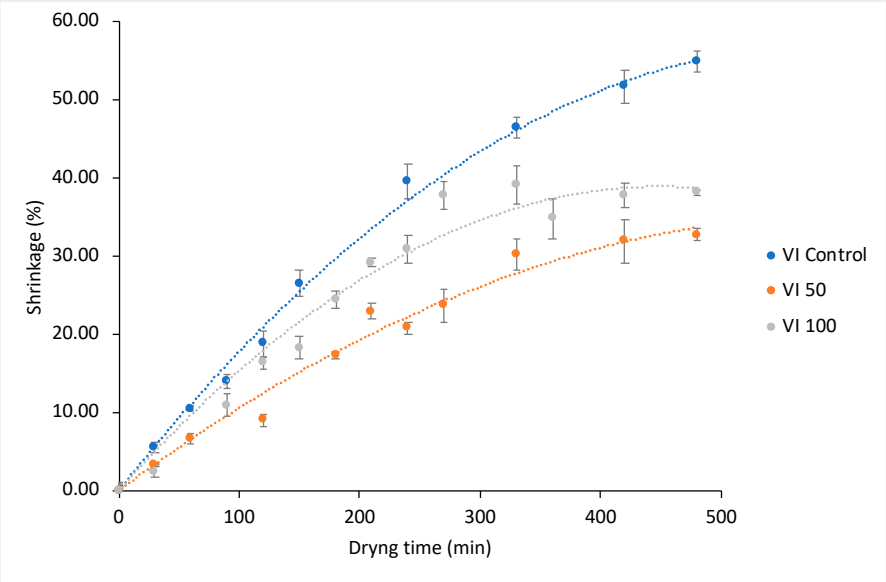


**Figure 3.** Shrinkage (%) during drying of vacuum impregnated apple samples.

Finally, the correlation between the Shrinkage and Moisture Ratio was investigated. The Pearson correlation coefficient (95% of confidence) was −0.997, −0.967 and −0.985 for the VI Control, VI 50 and VI 100 sample, respectively. The $p$-value < 0.05 indicated a statistical significative correlation between the two variables. The linear regression results are presented in Figure 4 for each sample.

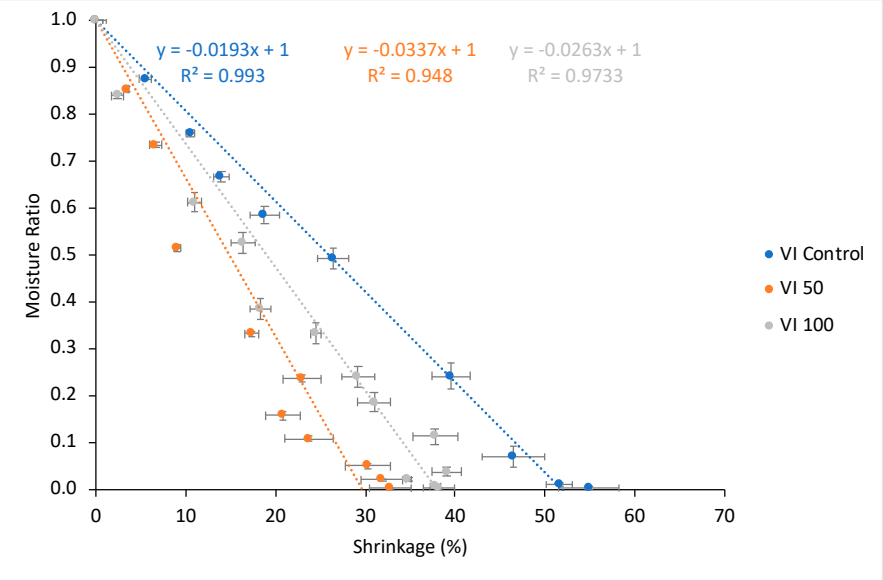


**Figure 4.** Linear regression for Shrinkage versus Moisture Ratio.

It should be noted that the methodology proposed to monitor the drying process does not consider the thickness of the sample. While the measurement of the real deformation is difficult from a technical point of view for industrial implementation, the bidimensional simplified method proposed in this study gives fast and simpler results in terms of data acquisition, processing and modelling. Besides, the thickness of the sample (fresh and VI = 5 mm; dried = 1–2 mm) is negligible with respect to the diameter of the sample (60 mm), thus the more important shrinkage phenomena take place on the diameter axis.

## 4. Conclusions

This study demonstrates the possibility of obtaining an upcycled food (apple dried snack) in a sustainable way, by enriching the apple matrix with blueberry juice, obtained from blueberry wastes.

Four applied mathematical models successfully fitted the experimental drying data. The drying kinetics of apple samples impregnated with trehalose blueberry juice were faster when compared to the control sample, making the drying process more sustainable. The effect of trehalose on the stability of anthocyanin was also assessed. Samples impregnated with blueberry juice and 100 g/kg of trehalose retained near four times more anthocyanin when compared to the control sample. The usefulness of direct formulation using VI technology to formulate new foods with increased stability and nutritional value was demonstrated. Besides, by means of image analysis and product shrinkage evaluation, it was possible to model the moisture decrease rate of each sample, providing a non-destructive and easy methodology to monitor the drying process.

Summarizing, the proposed formulation and methodology allow a more sustainable development and production of a fruit-based snack, by reducing energy consumption because of the shorter drying time achieved with the addition of trehalose. Moreover, the inclusion of trehalose also increased the retention of antioxidant compounds and, therefore, improved the health-promoting status.

**Author Contributions:** Conceptualization, J.M.C. and S.T.; methodology, J.M.C., S.T. and U.T.; software, J.M.C.; validation, S.T., U.T. and S.R.; formal analysis, J.M.C.; investigation, J.M.C.; resources, P.R.; data curation, J.M.C.; writing—original draft preparation, J.M.C.; writing—review and editing, S.T., U.T., S.R., P.R. and M.D.R.; visualization, J.M.C.; supervision, M.D.R.; project administration, U.T. and P.R.; funding acquisition, P.R. All authors have read and agreed to the published version of the manuscript.

**Funding:** The authors acknowledge the financial support for this research provided by transnational funding bodies, partners of the H2020 ERA-NETs SUSFOOD2 and CORE Organic Cofunds, under the Joint SUSFOOD2/CORE Organic Call 2019 (MILDSUSFRUIT).

**Conflicts of Interest:** The authors declare no conflict of interest.

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