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Research article

Monitoring the metabolite content of seasoned zucchinis during storage by NMR-based metabolomics

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ABSTRACT

This study applied ^{1}H NMR metabolomics to monitor the metabolite content of baked and seasoned zucchinis packaged in both compostable and plastic trays. Polar extracts of samples stored at 4 °C up to 35 days were investigated to check for metabolite changes upon shelf life. The evolution of the integral values of only the main metabolites responsible for sample differentiation (lactate, acetate, malate, α and β glucose and sucrose) were further analysed and compared. In particular, the evaluation of lactate and acetate amount, considered markers of fermentation progress, showed a comparable performance for the two types of packaging in preserving the freshness of seasoned zucchinis, confirming the maintenance of food product composition within the declared shelf life period in the recommended storage conditions. Albeit preliminary, the results support the NMR metabolomics as a tool for identifying candidate metabolites to monitor the shelf life of foods, thereby improving the understanding of molecular changes during storage.

1. Introduction

Zucchini (*Cucurbita pepo* L.), belonging to the Cucurbitaceae family, is a common vegetable widely cultivated in temperate areas in Europe, America and Asia. These vegetables can be found in different shapes, from spherical to elongated, and can vary in skin colour from dark to light green, sometimes with fine white mottling or stripes. Zucchini is characterised by its low caloric content and exceptionally high water content. From a nutritional standpoint, zucchinis are rich in carbohydrates, minerals and vitamins (especially vitamin C and potassium) and phenolic compounds resulting in good nutritional additives to a balanced and healthy diet [1–3]. Zucchinis are consumed fresh with skin in salads or served cooked in soups or other recipes; moreover, at present, they are also delivered as a 'ready-to-eat' (RTE) product. RTE products are defined by the European Food Safety Authority as a 'food intended by the producer for direct consumption without the need for cooking or other processing'. RTEs are widespread because of their practicality, minimal preparation time and ease of consumption, and their safety is guaranteed by controlled production processes and protective packaging [4]. In particular, zucchinis as well as other RTE vegetables, are delivered in fresh, cooked and seasoned forms. Shelf life (SL), conceived as the time during which the food remains safe under well-defined storage conditions, maintaining the desired sensory, chemical, physical and biological characteristics in compliance with the label declaration, is applied also to RTE products [5]. Numerous factors could influence the SL, such as temperature and humidity changes, light exposure, gas transmission and risk of contamination by microorganisms and spores. Packaging plays a determinant role in extending the SL of food products, preventing or mitigating the environmental effects. Recently, the European Union regulations promoted a growing interest in bio-based materials

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production as substitutes for traditional petro-plastics, aiming to mitigate accumulation and environmental pollution. Meanwhile, analytical techniques such as NMR spectroscopy have seen increasing application in monitoring the SL of foods due to their pivotal role in food metabolomics studies [6,7]. The factors affecting SL impact the metabolite content of food, and in this regard, NMR combined with chemometrics was already successfully applied in the study of SL. Edwards et al. [8] evaluated time and temperature effects on the metabolite profile of milk during storage to identify possible candidate compounds for monitoring SL. The same approach was adopted to predict the mozzarella cheese SL, submitted to irregular refrigeration practices [9]. Other studies were focused on the evaluation of SL of different fishes [10–12] and meat types [13]. Concerning fruits and vegetables, the SL of fresh cantaloupe juice and pumpkin has been evaluated [14,15]; in particular, the NMR metabolite profiling of fermented cantaloupe juice was investigated evaluating also different ratios of fresh and fermented cantaloupe juice, while a multidisciplinary approach, including the NMR-based metabolomics, was performed to evaluate the preservation of fresh pumpkin using three commercial polymeric films. Finally, in our previous study, the NMR metabolomics indicated that saffron produced under optimum processing and appropriate storage conditions, maintained its valuable characteristics for up to 4 years, indicating its freshness [16]. Concerning zucchinis, some studies focus on the possibility of extending their SL improving the conservation with different approaches. For example, the effect of different coatings on the quality of fresh zucchini during storage was investigated by evaluating physicochemical, visual [17] and sensorial properties [18]. The effects of individually shrink wrapping zucchini fruits on different quality parameters, namely cold tolerance, chilling injury, the loss of weight and firmness during storage at 4 °C, were also investigated [19] as well as the effects of other different treatments on fresh zucchini. In this context, the dipping treatments were evaluated by investigating the effect on sensorial and microbiological parameters [1]; a pre-storage treatment with nitric oxide on the chilling tolerance during post-harvest conservation at low temperature [20] and the exposure to ozone on the quality of zucchini [21] were also evaluated. In another study, the effects of different packaging films on the SL and the post-harvest quality of different zucchini cultivars, at two maturity stages, were investigated by monitoring physicochemical parameters, nutritional composition and sensorial characters [22]. The effect of high pressure processing during refrigerated storage on the stability of RTE foods, including zucchini, was investigated by monitoring microbial loads and physico-chemical and sensorial properties [23]. Finally, Anacarso et al. [24] evaluated the use of chitosan and Enterocin 416K, alone or in combination, as a feasible solution to obtain microbiological safety and to extend the SL of artificially contaminated RTE vegetables.

To the best of our knowledge, this is the first application of NMR metabolomics in monitoring metabolite content of RTE food, consisting of baked and seasoned zucchinis, during refrigerated storage. In particular, the combined use of NMR spectroscopy and chemometrics has been applied to monitor the metabolite content of baked and seasoned zucchinis packaged in both compostable and plastic trays and stored at 4 °C for up to 35 days. The metabolomic analysis can help in identifying candidate metabolites that are useful to estimate the SL of the product, improving the understanding of molecular changes during storage. Moreover, the results allowed to evaluate the performance of the two types of packaging in preserving the freshness of baked and seasoned zucchinis.

Table 1
List of baked and seasoned zucchini samples analysed with the indication of sampling, days of storage and type of packaging.

Sampling[a]	Sample	Storage (days)	Packaging
1	0P	0	Plastic
1	3P	3	Plastic
1	7P	7	Plastic
1	10P	10	Plastic
1	14P	14	Plastic
1	17P	17	Plastic
1	22P	22	Plastic
1	24P	24	Plastic
1	27P	27	Plastic
1	30P	30	Plastic
1	31P	31	Plastic
1	35P	35	Plastic
1	0C	0	Compostable
1	3C	3	Compostable
1	7C	7	Compostable
1	10C	10	Compostable
1	14C	14	Compostable
1	17C	17	Compostable
1	22C	22	Compostable
1	24C	24	Compostable
1	27C	27	Compostable
1	30C	30	Compostable
1	31C	31	Compostable
1	35C	35	Compostable
2	7C_2	7	Compostable
2	14C_2	14	Compostable
2	22C_2	22	Compostable
2	35C_2	35	Compostable

[a] 1 = sampling in February 2021, 2 = sampling in June 2021.

2. Methods and materials

2.1. Sample preparation

In total, 28 baked zucchini samples, seasoned with EVO oils and a mixture of spices, were provided by Mirtilla Bio (Grandate, Como, Italy). The first set (February 2021) included 12 samples packaged in compostable (PLA, polylactic acid) trays and 12 samples packaged in plastic (PP, polypropylene) trays, all in a modified atmosphere (CO_2/N_2 80/20). Each tray contained 200 g of chopped baked and seasoned zucchinis. All trays were heat-sealed with a film (a blend of PLA and modified cellulose), and the samples were stored at 4 °C. The producer suggested an SL of 21 days. Accordingly, the sampling was performed to include this period of time and continued until 35 days. Reasonably, after 35 days, the product would no longer be commercialised due to the metabolic/fermentative modifications. At each established sampling point (t = 0, 3, 7, 10, 14, 17, 22, 24, 27, 30, 31 and 35 storage days), after accurately mixing the content of each tray, 10 g of chopped zucchinis were lyophilised and stored at −80 °C until NMR analysis. The second set (June 2021) of four zucchini samples packaged only in compostable trays was provided to check the reproducibility of the results obtained on the first set. These samples were managed as previously described; all sample details are reported in Table 1. A biphasic extraction was performed at room temperature in glass vials by adding 500 μL of deuterated water buffered at pH = 7 (D_2O, Merck, 99.96 atom % D, Milan, Italy), 500 μL of deuterated methanol (CD_3OD, Eurisotop, 99.96 atom % D, Saint Aubin, France) and 750 μL of deuterated chloroform ($CDCl_3$, Merck, 99.96 atom % D, Milan, Italy) to 40 mg of lyophilised and ground seasoned zucchinis. The samples were vortexed, and centrifuged after 30 min at 3500 rcf for 10 min at 4 °C. A total of 500 μL of polar extract was used for NMR

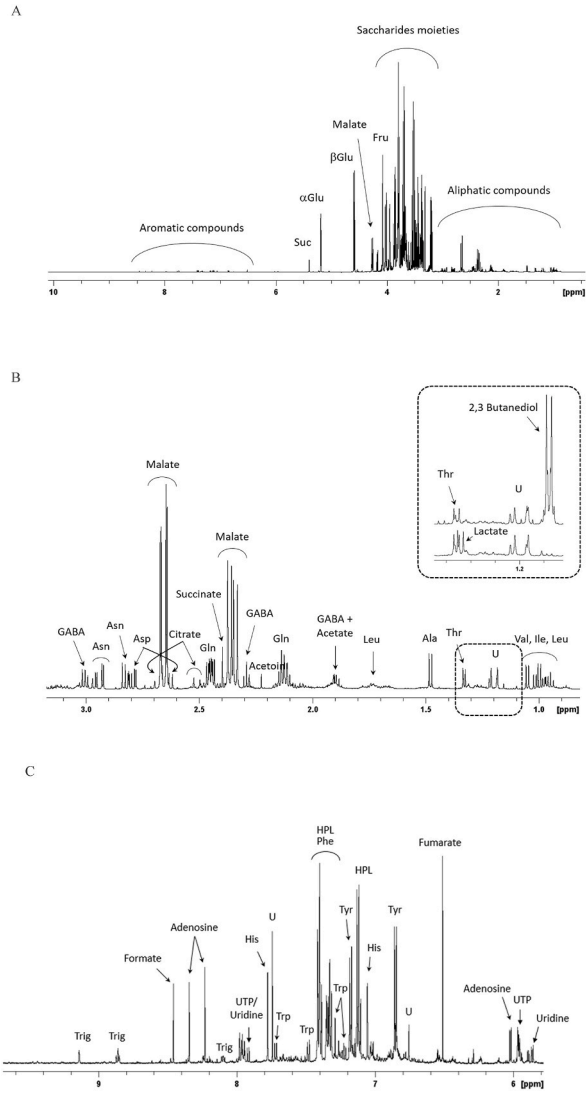

Fig. 1. Representative ^{1}H NMR spectrum of polar extract of baked and seasoned zucchinis (A) with the expansion of aliphatic (B) and aromatic (C) regions. The assignments of the main metabolites are reported.

analysis, and the hydrophobic phase was discarded.

2.2. NMR data acquisition and processing

All NMR spectra were recorded on Bruker AVANCE NEO 600 spectrometer (Bruker Biospin, GmbH Rheinstetten, Karlsruhe, Germania), operating at 14.07 T, equipped with a 5 mm reverse Z gradient cryoprobe *Prodigy*, and thermostated autosampler, at 298 K. Monodimensional ^{1}H experiments were acquired with a solvent presaturation scheme with 128 scans over 64K of data, and a spectral width of 7143Hz was employed. A total relaxation time of 32 s was used to allow the complete relaxation of all nuclei. A resolution enhancement function (LB = 0.3 Hz) was applied before Fourier transformation. All spectra were phased, baseline corrected and aligned with respect to α-glucose anomeric proton occurring at 5.19 ppm. After the exclusion of residual solvents signals in the range of 4.76–4.90 ppm (water), and 3.29–3.33 ppm (methanol), spectra were subjected to manual bucketing in the interval of 0.10–10.00 ppm according to the resonances assignment. For each spectrum, the buckets normalization was performed with respect to the total integral value set to 100, using the ACD/Spec Manager (ACD Labs, version 11, Toronto, Ontario Canada). Bidimensional ^{1}H–^{13}C HSQC, and ^{1}H–^{1}H TOCSY spectra have been recorded with the same spectral width parameters of the monodimensional spectra, by using 256 scans.

2.3. Statistical methods

The NMR data matrix was imported into SIMCA-P 13.03 (Sartorius Data Analytics, Umeå, Sweden) for principal component analysis (PCA) by using 'mean centring' as data pretreatment.

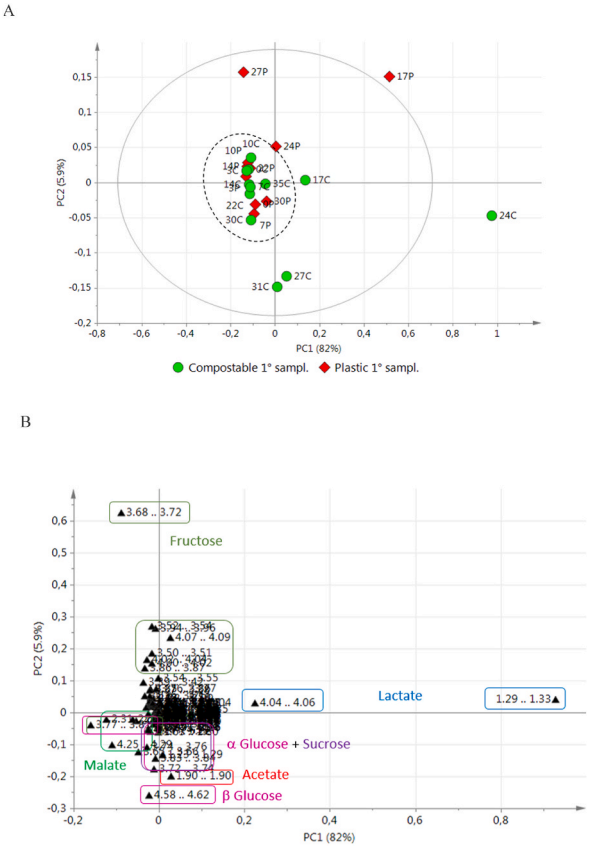

Fig. 2. Score scatter plot (A) and loading plot (B) of PCA performed including all zucchinis analysed of 1st sampling (February 2021) with the exclusion of 31P and 35P resulted strong outliers. Green filled circles, and red diamonds represent zucchinis packaged in compostable and plastic trays, respectively. The main metabolites responsible of samples differentiation are highlighted in the loading plot. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3. Results and discussion

3.1. NMR spectra analysis

The biphasic extraction was performed to exclude all possible amphiphilic components from the polar extracts as well as fatty acids and olive oil components. The ^{1}H NMR spectrum of the polar extract of baked and seasoned zucchinis is represented in Fig. 1A–C. The assignment of resonances was performed with the use of spectra of reference compounds and confirmed with data from the existing literature [25], and the use of databases (HMBD, BMRB, Chenomx). The ^{1}H NMR spectrum is dominated by signals of α- and β-glucose anomerics at 5.19 ppm and 4.59 ppm, respectively), fructose at 4.02 ppm), malate at 4.27, 2.66 and 2.35 ppm and sucrose (anomeric proton at 5.40 ppm). Less intense resonances were present in the aliphatic (Fig. 1B) and aromatic (Fig. 1C) regions. In particular, these regions revealed the presence of organic acids, amino acids (aliphatics and aromatics) as well as alkaloids and nucleotides. All the recognised metabolites and the relative assignments (^{1}H and ^{13}C) are summarised in Table S1.

3.2. Multivariate statistical analysis of NMR data

3.2.1. First sampling

Initially, ^{1}H NMR data referring to all 24 zucchinis sampled in February 2021 were analysed. The PCA yielded two components, explaining 99.5% of the total variance, with $Q^2_{cum} = 72.9\%$. The score plot showed that samples with the longest SL packaged in plastic trays (35P and 31P), appeared as strong outliers; conversely, all other samples were grouped in the middle of the score plot, regardless of the packaging type. The corresponding loading plot highlighted a high content of lactate in sample 35P and a high content of 2,3 butanediol and succinate in sample 31P, suggesting that those samples have experienced lactic and most likely a butanediol fermentation processes, respectively [26,27]. All other samples' results were characterised by a higher amount of malate and

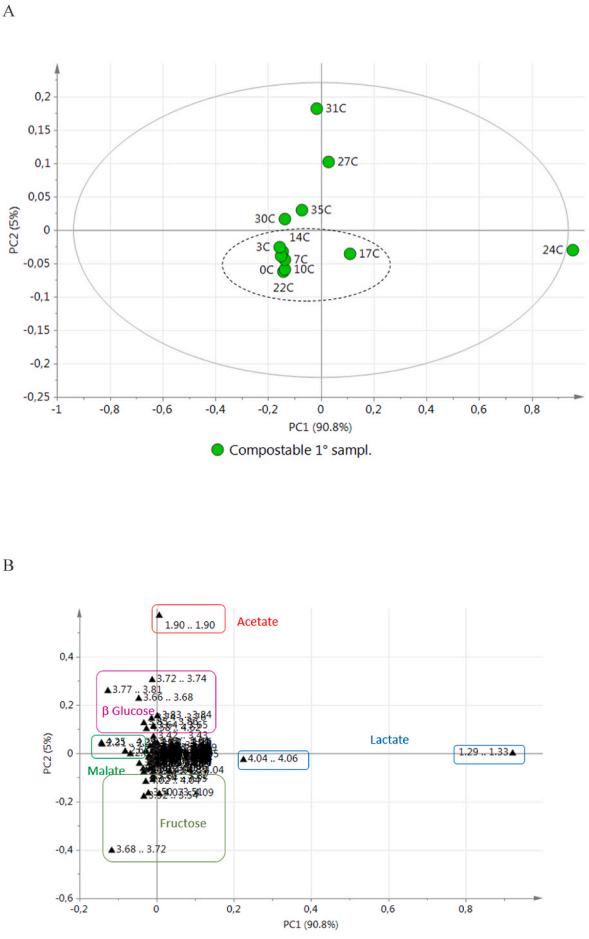

Fig. 3. Score scatter plot (A) and loading plot (B) of PCA performed considering all zucchinis of 1st sampling (February 2021) packaged in compostable trays. Samples stored up to 22 days are highlighted by dotted line. The main metabolites responsible of samples differentiation are highlighted in the loading plot.

α,β-glucose. The exclusion of 31P and 35P samples led to a new PCA (2PCs, $R^2X = 87.9\%$, $Q^2_{cum} = 24.4\%$), revealing a partial overlap of samples stored in plastic and in compostable trays at 0, 3, 7, 10, 14, 22 and 30 days of storage, along with sample 35C (Fig. 2A). These findings suggested that the preservation performance of the two types of packaging was comparable based on the metabolite content. Interestingly, samples stored for 17, 24 and 27 days, and sample 31C were scattered in other regions of the score plot. The corresponding loading plot (Fig. 2B) indicated lactate as the characteristic metabolite for samples 17P, 17C and 24C while acetate along with α- and β-glucose, sucrose and bucket at 1.23 ppm (not assigned yet) as the characteristic metabolites for samples 27C and 31C. The higher content of lactate and acetate in these samples suggested that fermentation processes occurred. Samples 24P and 27P showed instead a higher content in fructose most likely due to an intrinsic higher content of fructose in the vegetable fruits used for preparing those samples or due to less fermentative processes occurring during storage. Successively, zucchinis packaged in compostable and plastic trays were individually analysed for better monitoring the evolution of metabolite content during storage, according to the packaging used. The PCA performed on 12 zucchini samples packaged in compostable trays resulted in three components explaining 97.9% of the total variance, with $Q^2_{cum} = 62.7\%$. The score plot reported in Fig. 3A showed samples from 0 to 22 days of storage grouped together, suggesting an equivalent content of metabolites during storage, at least until 22 days. Zucchinis sampled at 24 days were characterised by a high content in lactate, while samples 27C and 31C, and to a lower extent, samples 30C and 35C, showed a higher content in acetate. Moreover, samples 30C and 35C were characterised by a higher content of β-glucose (loading plot in Fig. 3B). The higher content of organic acids could be ascribed to a mixed acid fermentation process started after 24 days of storage, with a production of lactate, and a successive production of acetate [28]. Interestingly, sample 17C showed a higher content in lactate with

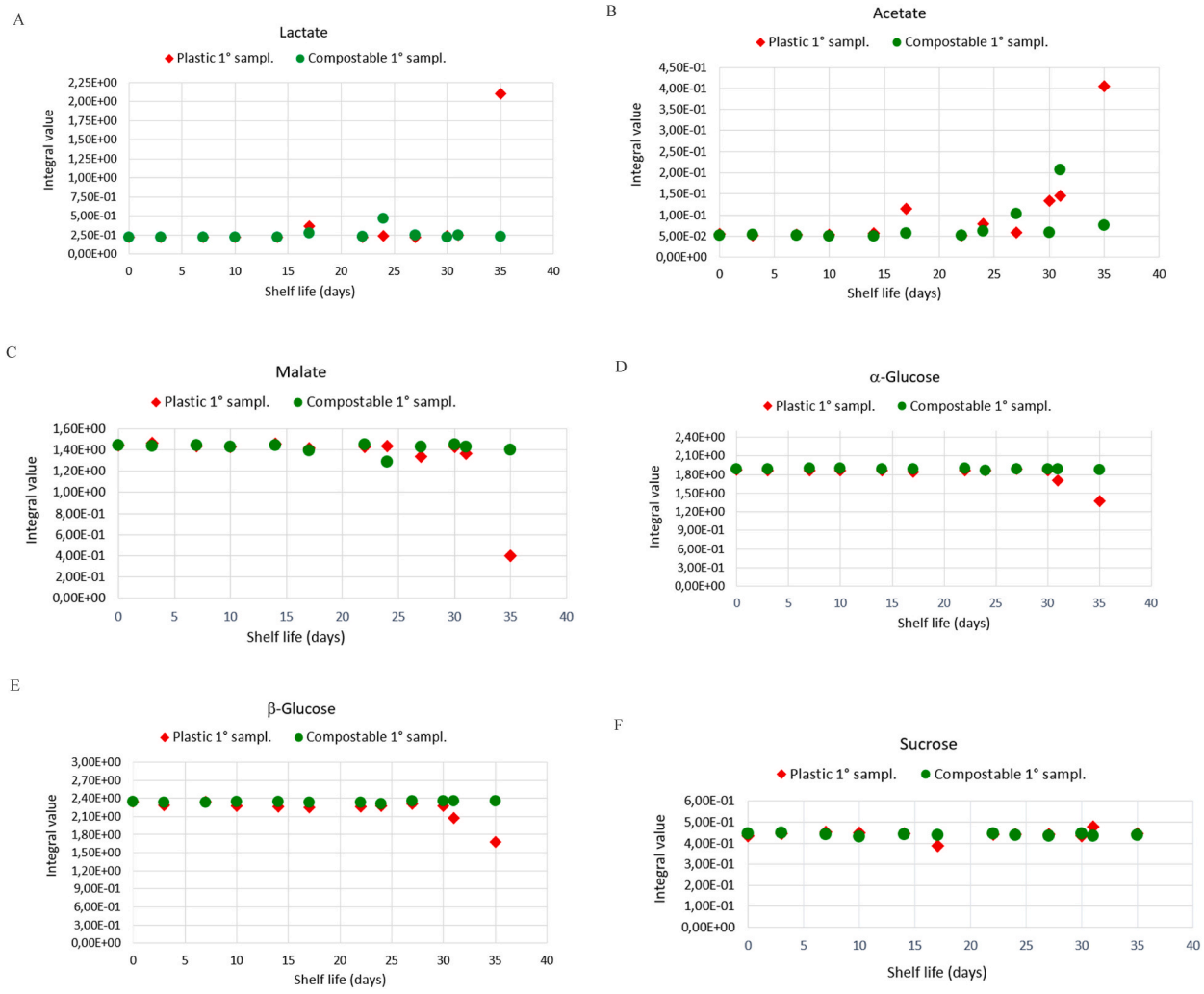

Fig. 4. The evolution of the main metabolites responsible of samples differentiation considering zucchinis of 1st sampling packaged both in plastic (red diamonds) and compostable (green filled circles) trays during storage, are reported. Particularly, the evolution of integrals values of buckets included lactate (bucket at 4.04 ppm, A), acetate (bucket at 1.90 ppm, B), malate (bucket at 4.25 ppm, C), α-glucose (bucket at 5.17 ppm, D), β-glucose (bucket at 4.58 ppm, E), and sucrose (bucket at 5.38 ppm, F) are reported. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

respect to all samples grouped in the middle of the score plot, and particularly higher than sample 22C of a longer storage time. Nevertheless, the lactate content of sample 17C was significantly lower than those observed in sample 24C.

The PCA performed considering the 12 zucchini samples packaged in plastic trays resulted in two principal components explaining 99.7 % of the total variance, with $Q^2_{cum} = 87.8\%$. The score plot reported in Fig. S1A showed samples grouped in the middle of the score plot with the exception of samples 31P and 35P, which resulted in outliers and were characterised by 2,3 butanediol and succinate, and lactate, respectively. All other samples' results were characterised by malate, and α,β glucose (Fig. S1B). After excluding 31P and 35P samples, a new PCA was performed (3PCs, $R^2X = 94.8\%$, $Q^2_{cum} = 44.4\%$). The score plot reported in Fig. S2A showed a grouping for all samples with the exception of 17P, which resulted in enriched lactate, suggesting that a fermentation process occurred, and sample 27P mainly characterised by fructose, α and β glucose (Fig. S2B). Interestingly sample 30P, with a longer storage time, presented a metabolite content comparable with those of all other samples with shorter storage grouped in the left down side of the score plot, and characterised by malate, sucrose and glutamine.

The evolution of the main metabolites, responsible for sample differentiation considering zucchinis packaged both in plastic and compostable trays, was further evaluated and compared taking into account the evolution of the corresponding integral values (Fig. 4A–F). The lactate content (Fig. 4A) was equivalent and constant from 0 to 14 days of storage for samples packaged in both plastic or compostable trays. A small increment was observed for sample 17C and slightly more pronounced for sample 17P; its value was nevertheless returning almost to the initial values at 22 days of storage for zucchinis packaged in both trays type. After 22 days some little increments with respect to initial values were observed in sample 24C, and to a great extent in sample 35P. The acetate content (bucket integral value at 1.90 ppm, Fig. 4B) remained constant for zucchinis packaged in both tray types until 22 days of storage, with the exception of sample 17P, which showed an increment most likely due to a fermentation process; in fact, this sample showed also a higher content of lactate then samples with shorter storage period as reported above (Fig. 4A). After 22 days of storage, a significant variability of acetate content was observed in all zucchinis irrespectively the packaging type. Analogously to lactate, a great amount of acetate was observed in the 35P sample. The malate content (bucket integral at 4.25 ppm, Fig. 4C) remained almost constant for all zucchinis until 22 days of storage; afterwards, a little decrease was randomly observed with a great reduction only for sample 35P. The content of α,β glucose (Fig. 4D and E) showed a constant value for all samples until 30 days of storage, and only zucchinis packaged in

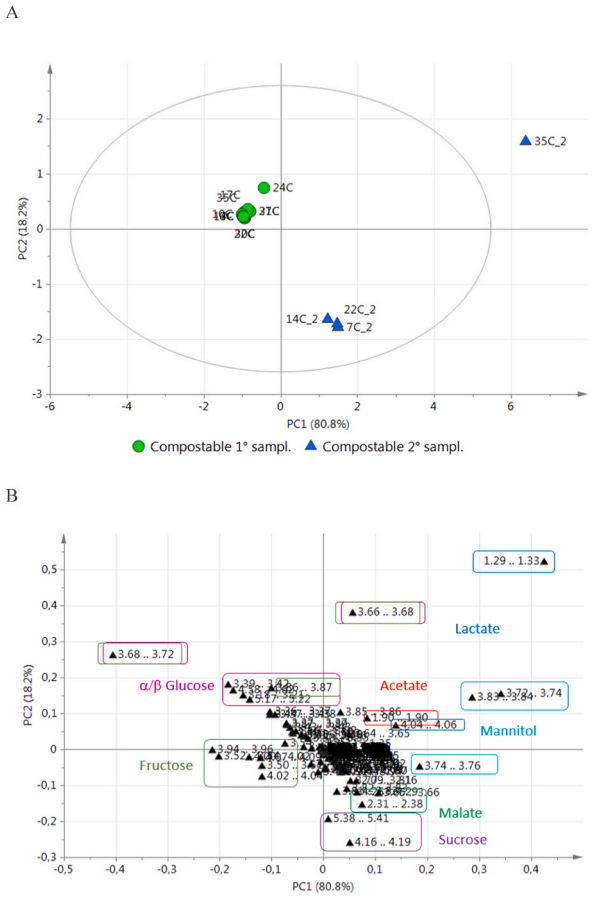

Fig. 5. Score scatter plot (A) and loading plot (B) of PCA performed considering zucchinis of both 1st (green filled circles) and 2nd (blue triangles) sampling packaged in compostable trays. The main metabolites responsible of samples differentiation are highlighted in the loading plot. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

plastic trays showed a smooth decrease in 31P and more defined in 35P samples. Finally, the sucrose content (bucket integral value at 5.38 ppm, Fig. 4F) remained almost constant for all samples during all storage periods, with the exception of a little decrease for sample 17P confirming a more intense fermentation process with respect to other samples, and a little increase for sample 31P. These results showed the performance in the preservation of seasoned zucchinis, almost comparable for plastic and compostable trays, up to 22 days of storage. After this period, fermentation processes took place, leading to a random increase of lactate and acetate contents, with a notable evolution in sample 35P, which showed a concomitant reduction of α,β-glucose content. Anomalous behaviour was observed for samples at 17 days of storage, mostly for those packaged in plastic trays, which showed a higher increment in the above-mentioned organic acids, suggesting that some fortuitous modifications in the preservation of zucchinis occurred. In the end, opting for compostable trays was a good choice, combining more sustainable packaging with effective capabilities in preserving seasoned zucchinis, guaranteeing a SL until 22 days from the metabolic point of view, in contrast to using plastic trays, which involve more expensive disposal processes.

3.2.2. Second sampling

To verify the results observed in the first sampling, a second set of seasoned zucchinis was investigated in June 2021. In particular, the metabolite content was monitored to check whether the stability of the metabolome of seasoned zucchinis until 22 days of storage was effective by considering only samples packaged in compostable trays, currently used by the producer. As reported in Table 1, the sampling was performed after 7, 14, 22 and 35 days of storage. In fact, considering the evolution of the metabolites in the first sampling, a constant trend was observed for the first 14 days, therefore the sampling was reduced for the second one checking the metabolite content after 7 and 14 days. Another sampling was considered at 22 days, close to the shelf life suggested by the producer, while the last one was at 35 days, largely over the suggested SL. The initial PCA was performed on the NMR data referring to all zucchinis sampled in February 2021 and June 2021. Four components explained 99.9% of the total variance, with $Q^2_{cum} = 98.7\%$. The score plot (Fig. 5A) showed a clear grouping of zucchinis according to the sampling. In particular, the zucchinis of the first sampling clustered in the upper left-hand side of the score plot resulting characterised by a higher content of α,β glucose and fructose, while zucchinis of the second sampling grouped in the lower right-hand side with the exception of sample 35C_2, which resulted as a strong outlier characterised by a high content of lactate, acetate and mannitol, products derived from fermentation processes [29]. All the other zucchinis of the second sampling were enriched in sucrose and malate (Fig. 5B). The new PCA performed, excluding the strong outlier sample (35C_2) and considering zucchinis of the two samplings, with the same period of storage (7, 14 and 22 days, Figs. S3A and S3B), confirmed an intrinsic difference in the metabolite content, according to sampling, and specifically concerning the

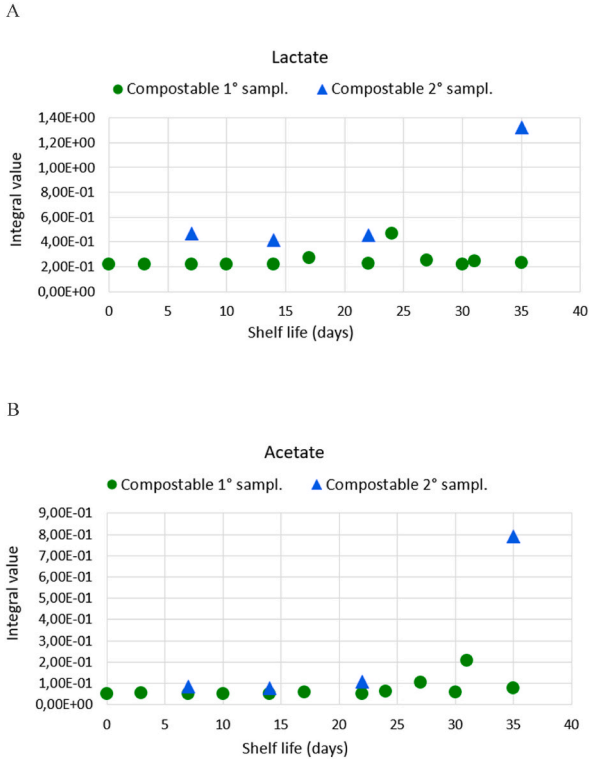

Fig. 6. The evolution of the integrals values of buckets including lactate (bucket at 4.04 ppm, A), and acetate (bucket at 1.90 ppm, B) during the storage of zucchinis packaged in compostable trays of 1st sampling (green filled circles) and 2nd sampling (blue triangles) are reported. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

saccharides content. These differences could be attributed to the different periods of harvest of zucchinis. Successively, the lactate and acetate content of all zucchinis packaged in compostable trays was monitored and compared. As reported in Fig. 6A, the lactate content remained almost constant until 22 days of storage for both samplings, even if the zucchinis of the second sampling showed an intrinsic higher value. The same evolution was observed for acetate, as reported in Fig. 6B. Only the 35C_2 sample showed a very high content of lactate and acetate, due to a fermentation processes occurred. In fact, the same sample showed a significant decrease in the sucrose content (Fig. S4).

4. Conclusion

The findings suggest that baked and seasoned zucchinis packaged in compostable and plastic trays showed comparable performance in conservation until 22 days of storage at 4 °C, according to the metabolite content. In addition, even if an intrinsic difference in metabolite content was evident, particularly involving the saccharides content in zucchinis packaged in compostable trays and sampled in two different periods, a constant content in lactate and acetate was observed until 22 days of storage, suggesting that up to this storage period, no significant fermentation process occurred that can alter the metabolite content of the product. These results, even if preliminary, confirm the potentiality of NMR metabolomics in finding candidate metabolites useful for estimating the SL of foods, thereby improving the understanding of molecular changes during storage, such as those arising from fermentation or degradation processes. The combining of NMR metabolomics results with sensorial tests and microbiological analysis may provide a valid tool to accurately evaluate both quality and the freshness of foods. Further studies will certainly consolidate these findings.

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CRediT authorship contribution statement

L.R. Cagliani: Writing – review & editing, Validation, Methodology, Formal analysis, Conceptualization. **R. Consonni:** Writing – review & editing, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing 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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e25976.

References

[1] W. De Bruin, W. Rossouw, L. Korsten, Comparison of safe alternative dipping treatments to maintain quality of zucchini, J. Food Qual. 39 (2016) 109–115, https://doi.org/10.1111/jfq.12186.
[2] L. Tejada, L. Buendía-Moreno, A. Villegas, J.M. Cayuela, E. Bueno-Gavilá, P. Gómez, A. Abellán, Nutritional and sensorial characteristics of zucchini (*Cucurbita pepo* L.) as affected by freezing and the culinary treatment, Int. J. Food Prop. 23 (2020) 1825–1833, https://doi.org/10.1080/10942912.2020.1826512.
[3] I. Iswaldi, A.M. Gómez-Caravaca, J. Lozano-Sánchez, D. Arráez-Román, A. Segura-Carretero, A. Fernández-Gutiérrez, Profiling of phenolic and other polar compounds in zucchini (*Cucurbita pepo* L.) by reverse-phase high-performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry, Food Res. Int. 50 (2013) 77–84, https://doi.org/10.1016/j.foodres.2012.09.030.
[4] F. Sgroi, F. Piraino, E. Donia, Determinants of ready-to-eat products purchase intentions: an empirical study among the Italian consumers, Hortscience 53 (2018) 656–660, https://doi.org/10.21273/HORTSCI12834-17.
[5] N.W.G. Young, G.R. O'Sullivan, The influence of ingredients on product stability and shelf life, in: David Kilcast, Persis Subramaniam (Eds.), Woodhead Publishing Series in Food Science, Technology and Nutrition, Food and Beverage Stability and Shelf Life, Woodhead Publishing, 2011, pp. 132–183, https://doi.org/10.1533/9780857092540.1.132 *(Chapter 5)*.
[6] R. Consonni, L.R. Cagliani, in: Steve L. Taylor (Ed.), Chapter 4 - Nuclear Magnetic Resonance and Chemometrics to Assess Geographical Origin and Quality of Traditional Food Products, vol. 59, Academic Press, Adv. Food Nutr. Res, 2010, pp. 87–165, https://doi.org/10.1016/S1043-4526(10)59004-1.
[7] S. Li, Y. Tian, P. Jiang, Y. Lin, X. Liu, H. Yang, Recent advances in the application of metabolomics for food safety control and food quality analyses, Crit. Rev. Food Sci. Nutr. 61 (2021) 1448–1469, https://doi.org/10.1080/10408398.2020.1761287.

[8] K.M. Edwards, A. Badiger, D.R. Heldman, M.S. Klein, Metabolomic markers of storage temperature and time in pasteurized milk, Metabolites 11 (2021) 419, https://doi.org/10.3390/metabo11070419.
[9] F.C. Gonçalves, V.M. de Oliveira, F.T. Martins, L.M. Lião, P.H. Ferri, L.H.K. Queiroz Júnior, Predicting chemical shelf life of mozzarella cheese submitted to irregular refrigeration practices by Nuclear Magnetic Resonance spectroscopy and statistical analysis, J. Food Compos. Anal. 105 (2022) 104229, https://doi.org/10.1016/j.jfca.2021.104229.
[10] C. Heude, K. Elbayed, T. Jezequel, M. Fanuel, R. Lugan, D. Heintz, P. Benoit, M. Piotto, Metabolic characterization of caviar specimens by ^{1}H NMR spectroscopy: towards caviar authenticity and integrity, Food Anal. Methods 9 (2016) 3428–3438, https://doi.org/10.1007/s12161-016-0540-4.
[11] E. Jääkeläinen, L.M.A. Jakobsen, J. Hultman, N. Eggers, H.C. Bertram, J. Björkroth, Metabolomics and bacterial diversity of packaged yellowfin tuna (*Thunnus albacares*) and salmon (*Salmo salar*) show fish species-specific spoilage development during chilled storage, Int. J. Food Microbiol. 293 (2019) 44–52, https://doi.org/10.1016/j.ijfoodmicro.2018.12.021.
[12] E.M. Kirkholt, A. Dixiy, E. Shumilina, Changes in the composition of Atlantic salmon upon the brown seaweed (*Saccharina latissima*) treatment, Foods 8 (2019) 625, https://doi.org/10.3390/foods8120625.
[13] B.J. Muhialdin, H. Kadum, S. Fathallah, A.S.M. Hussin, Metabolomics profiling and antibacterial activity of fermented ginger paste extends the shelf life of chicken meat, Food Sci. Technol. 132 (2020) 109897, https://doi.org/10.1016/j.lwt.2020.109897.
[14] B.J. Muhialdin, H. Kadum, A.S.M. Hussin, Metabolomics profiling of fermented cantaloupe juice and the potential application to extend the shelf life of fresh cantaloupe juice for six months at 8°C, Food Control 120 (2021) 107555, https://doi.org/10.1016/j.foodcont.2020.107555.
[15] G. Di Matteo, P. Di Matteo, M. Sambucci, J. Tirillò, A.M. Giusti, G. Vinci, L. Gobbi, S.A. Prencipe, A. Salvo, C. Ingallina, M. Spano, A.P. Sobolev, N. Proietti, V. Di Tullio, P. Russo, L. Mannina, M. Valente, Commercial bio-packaging to preserve the quality and extend the shelf life of vegetables: the case-study of pumpkin samples studied by a multimethodological approach, Foods 10 (2021) 2440, https://doi.org/10.3390/foods10102440.
[16] S.A. Ordoudi, L.R. Cagliani, S. Lalou, E. Naziri, M.Z. Tsimidou, R. Consonni, ^{1}H NMR-based metabolomics of saffron reveals markers for its quality deterioration, Food Res. Int. 70 (2015) 1–6, https://doi.org/10.1016/j.foodres.2015.01.021.
[17] R. Jafari, M. Zandi, A. Ganjloo, Effect of gelatin-alginate coating containing anise (*Pimpinella anisum* L.) essential oil on physicochemical and visual properties of zucchini (*Cucurbita pepo* L.) fruit during storage, J. Food Process. Preserv. 46 (2022) e16756, https://doi.org/10.1111/jfpp.16756.
[18] I. Bleoanca, A. Lanciu, L. Patrascu, A. Ceoromila, D. Borda, Efficacy of two stabilizers in nanoemulsions with whey proteins and thyme essential oil as edible coatings for zucchini, Membranes 12 (2022) 326, https://doi.org/10.3390/membranes12030326.
[19] Z. Megías, C. Martínez, S. Manzano, A. García, M. del Mar Rebolloso-Fuentes, D. Garrido, J.L. Valenzuela, M. Jamilena, Individual shrink wrapping of zucchini fruit improves postharvest chilling tolerance associated with a reduction in ethylene production and oxidative stress metabolites, PLoS One 10 (2015) e0133058, https://doi.org/10.1371/journal.pone.0133058.
[20] R. Jiménez-Muñoz, F. Palma, F. Carvajal, A. Castro-Cegrí, A. Pulido, M. Jamilena, C. Romero-Puertas, D. Garrido, Pre-storage nitric oxide treatment enhances chilling tolerance of zucchini fruit (*Cucurbita pepo* L.) by S-nitrosylation of proteins and modulation of the antioxidant response, Postharvest Biol. Technol. 171 (2021) 111345, https://doi.org/10.1016/j.postharvbio.2020.111345.
[21] M. Glowacz, R. Colgan, D. Rees, Influence of continuous exposure to gaseous ozone on the quality of red bell peppers, cucumbers and zucchini, Postharvest Biol. Technol. 99 (2015) 1–8, https://doi.org/10.1016/j.postharvbio.2014.06.015.
[22] M.T. Blanco-Díaz, A. Pérez-Vicente, R. Font, Quality of fresh cut zucchini as affected by cultivar, maturity at processing and packaging, Packag. Technol. Sci. 29 (2016) 365–382, https://doi.org/10.1002/pts.2214.
[23] R. Masegosa, J. Delgado-Adámez, R. Contador, F. Sánchez-Íñiguez, R. Ramírez, Effect of processing by hydrostatic high pressure of two ready to heat vegetable meals and stability after refrigerated storage, Food Sci. Technol. Int. 20 (2014) 605–615, https://doi.org/10.1177/1082013213497493.
[24] I. Anacarso, S. de Niederhäusern, R. Iseppi, C. Sabia, M. Bondi, P. Messi, Anti-listerical activity of chitosan and Enterocin 416K in artificially contaminated RTE products, Food Control 22 (2011) 2076–2080, https://doi.org/10.1016/j.foodcont.2011.06.001.
[25] A.C. Abreu, L.M. Aguilera-Sáez, A. Peña, M. García-Valverde, P. Marín, D.L. Valera, I. Fernández, NMR-Based metabolomics approach to study the influence of different conditions of water irrigation and greenhouse ventilation on zucchini crops, J. Agric. Food Chem. 66 (2018) 8422–8432, https://doi.org/10.1021/acs.jafc.8b02590.
[26] W. Meng, L. Zhang, M. Cao, Y. Zhang, Y. Zhang, P. Li, Z. Kang, S. Guo, P. Xu, C. Ma, C. Gao, 2,3-butanediol synthesis from glucose supplies NADH for elimination of toxic acetate produced during overflow metabolism, Cell Discov. 7 (2021) 43, https://doi.org/10.1038/s41421-021-00273-2.
[27] M. Köpke, C. Mihalcea, F.M. Liew, J.H. Tizard, M.S. Ali, J.J. Conolly, B. Al-Sinawi, S.D. Simpson, 2,3-butanediol production by acetogenic bacteria, an alternative route to chemical synthesis, using industrial waste gas, AEM 77 (2011) 5467–5475, https://doi.org/10.1128/AEM.00355-11.
[28] R. Di Cagno, R. Coda, M. De Angelis, M. Gobbetti, Exploitation of vegetables and fruits through lactic acid fermentation, Food Microbiol. 33 (2013) 1–10, https://doi.org/10.1016/j.fm.2012.09.003.
[29] J.W. Yun, S.C. Kang, S.K. Song, Mannitol accumulation during fermentation of *Kimchi*, J. Ferment. Bioeng. 81 (1996) 279–280, https://doi.org/10.1016/0922-338X(96)82224-0.