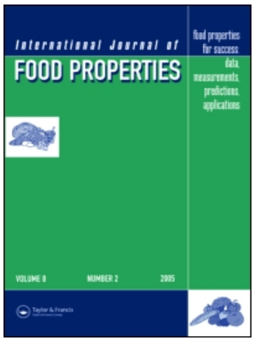

International Journal of Food Properties

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/ljfp20

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To cite this article: Luis Tejada , Laura Buendía-Moreno , Alba Villegas , José M. Cayuela , Estefanía Bueno-Gavilá , Pablo Gómez & Adela Abellán (2020) Nutritional and sensorial characteristics of zucchini (*Cucurbita pepo* L.) as affected by freezing and the culinary treatment, International Journal of Food Properties, 23:1, 1825-1833, DOI: 10.1080/10942912.2020.1826512

To link to this article: https://doi.org/10.1080/10942912.2020.1826512

Published online: 26 Oct 2020.

INTERNATIONAL JOURNAL OF FOOD PROPERTIES
2020, VOL. 23, NO. 1, 1825–1833
https://doi.org/10.1080/10942912.2020.1826512

Nutritional and sensorial characteristics of zucchini (*Cucurbita pepo* L.) as affected by freezing and the culinary treatment

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ABSTRACT
The objective of this study was to analyze the effect of freezing and different culinary treatments (stir-fried and steamed) on the nutritional composition, bioactive compounds, and sensorial characteristics of zucchini. The zucchini was stir-fried for 8 min at 250ºC and steamed for 13 min at 100ºC. The freezing of raw zucchini did not significantly affect its nutritional compounds. The stir-frying process provoked important moisture loss; this being higher when zucchini was previously frozen. The content of ash, proteins and fats significantly increased in stir-fried zucchini. There was an important decrease in antioxidant activity and phenolic compounds when the zucchini was frozen. The antioxidant activity was much greater in the stir-fried produce in comparison to steamed and raw. Freezing the raw zucchini provoked a significant loss in its adhesiveness qualities and hardness. A lower hardness and adhesiveness were also observed in frozen zucchini after steamed and stir-fried, these changes were not being observed in freezing. The sensorial characteristics of previously frozen zucchini were valued less by the consumer as well as those that were steamed and stir-fried. The culinary treatment affected the sensorial values less and only better were observed in fresh steamed zucchini than the stir-fried.

ARTICLE HISTORY
Received 16 June 2020
Revised 14 September 2020
Accepted 16 September 2020

KEYWORDS
Cucurbita pepo L.; culinary treatment; freezing; bioactive compounds; sensorial; nutritional composition

Introduction

Vegetables provide great health benefits. They help to hydrate our body, are high in minerals, vitamins, and fiber, and provide few calories. They contain substances with antioxidant action, and therefore, essential to our diet. Plant-based foods are made up of significant amounts of healthy bioactive compounds and in addition reduce the risk of cardiovascular and degenerative diseases, such as cancer.[1]

Zucchini (*Cucurbita pepo* L.) is a fruit belonging to the *Cucurbita* genus and the *Cucurbitaceae* family. This family is made up of 22 species; the 5 most cultivated being *C. maxima*, *C. moschata*, *C. pepo*, *C. ficifolia*, and *C. argyrosperma*.[2] According to the Ministry of Agriculture, Fisheries, and Food, the production of zucchini in Spain is 591,341 tons (t) and a cultivation area of 11,037 hectares (ha).[3] Zucchini is one of the lowest caloric content vegetables (14 kcal/100 g) and has the highest water content (96.5%). Its consumption covers the needs of vitamins and minerals, especially vitamin C and potassium, vitamin C being the most significant. The presence of mucilage gives it emollient properties on the digestive system and as an easy-to-digest food, it is suitable for those with digestive problems.[4]

The sensorial, chemical, and physical properties of vegetables are altered during the cooking process. From a nutritional point of view, most of the changes produced are related to the influence on the concentration and bioavailability of nutrients and bioactive compounds in vegetables.[5] To

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minimize loss of nutrient properties, the thermic treatment applied to each food should differ depending on its nature.[6] When the temperature of food rises, basic cooking processes occur such as the softening of fibers, dissolving of chemical compounds, binding of proteins, the release of juices, and changes in appearance and taste. Therefore, excessive cooking times and high temperatures cause negative effects in the quality of the food.[7] It has been shown that thermic treatment to food affects the nutritional and sensorial quality of vegetables like spinach, cauliflower, cabbage, etc.[8,9] Is also important to consider the changes in the physicochemical characteristics of vegetables during different cooking techniques, above all during the thermal treatment, since this can predict the losses and gains of the nutritional components of food.[10]

All nutritional changes produced by the different cooking methods are not considered in nutritional tables of foods and the diets carried out are based mainly on the composition of the raw horticultural products. In addition to this lack of information, not many studies have evaluated how cooking affects the nutritional characteristics of foods. In this way there are currently few works that deal with this question specifically in zucchini and no studies have been carried out that address this issue, despite being one of the most globally consumed vegetables.

New trends in food consumption show that the population is looking for healthier options with less aggressive conservation treatments of their nutrients. However, fruits and vegetables have a limited shelf life and the application of technological processes is necessary to reduce their deterioration.[11] The most common method being freezing, which causes the crystallization of the water found in food therefore making it a determining factor in the organoleptic quality of the frozen produce. The formation of ice can break the cellular structures of the food, triggering oxidation reactions that limit storage time due to the degradation of compounds such as vitamins.

There are few studies dealing with the effect of freezing on the nutritional and antioxidant characteristics of vegetables. In this sense, studies showed that the content of ascorbic acid in broccoli decreased significantly when it was stored at −25ºC after 12 days.[12] Other research verified that the vitamin C content was higher in raw and frozen beans and asparagus.[13,14] However, zucchini showed similar values of vitamin C in its raw and frozen state. During the freezing process, ice crystals are produced from the extracellular and intracellular water of the food itself, which can break down cell walls and consequently developing losses in the quality of the vegetable.[15] A research study confirms the favorable effect of blanching prior to the quick freezing of green beans, thus improving their texture.[16]

On the other hand, despite the V gamma industry using diced and frozen zucchini to make other products, there is no record of any study evaluating the effect of freezing on the nutritional and sensory characteristics of the fresh produce even once it is cooked. Studies have only been carried out on aubergine, potato, pumpkin, tomato,[6] broccoli, and sweet pepper.[17] As a result, there is a need to arise to study the influence of freezing on the nutritional and sensory characteristics and the texture of fresh zucchini after different culinary treatments. The aim is to determine the most appropriate culinary technique to minimize losses and establish the nutritional changes that occur to then be able to create a diet.

Materials and methods

Sample preparation

Fresh mature commercial zucchini was obtained from a frozen vegetable company in the Region of Murcia. In order to minimize the effects of environmental conditions, the produce was harvested between April and May and from plants grown in the same greenhouse. Three batches of 60 kilograms (kg) of zucchini cut into 10 × 10 mm cubes were obtained, of which half (30 kg) were subjected to a commercial freezing (at −18ºC after a previous blanching), and the other 30 kg were kept fresh. Aliquots of 1,000 grams (g) were taken from each of the 6 batches (3 frozen and 3 fresh) to carry out the different analyses in raw, fried, and steamed zucchini.

For the stir-frying process, 1,000 g of zucchini was cooked with 64 g of extra virgin olive oil, at a temperature of 250ºC for 8.5 min. To steam, 1,000 g of zucchini from each batch was placed in a Rational oven at 100ºC and 100% moisture (HR), for 13 min. The zucchini cubes were placed on a perforated gastronorm tray to collect the water that resulted from the cooking process, which did not fall on the samples. For the physicochemical characterization after determining the immediate parameters (moisture, texture, and sensory) the zucchini were homogenized in a Thermomix TM5 (Vorwek, Spain) and kept in aseptic bottles and refrigerated until the rest of the tests were carried out.

Physicochemical characterization

Moisture, ash content, proteins, and fat were determined in all the samples of raw, stir-fried, and steamed zucchini (fresh and frozen). The moisture was determined by the method established by AOAC.[18] The ash content was measured by incinerating the samples at a temperature of 550ºC.[18] The determination of proteins and fat was carried out using the technique Kjeldahl and Soxhlet, respectively.[18]

Analysis of bioactive compounds

Extraction of sample: The extraction was carried out with some modifications but in line with the Ferracane et al. 2008 method.[19] For this, 10 g of zucchini together with 40 mL of ethanol at 60% was centrifuged at 1,000 rpm for 5 min at room temperature, from which the precipitate was extracted. This process was repeated 4 times. The supernatant from all extractions was combined and vacuum dried at a temperature below 30°C and the residue obtained was again dissolved by ultrasonic stirring, to a final volume of 20 mL. The extracts were stored at −18ºC until it was time to analyze.

Phenolic Compounds Determination: Total phenolic content was measured using the Folin–Ciocalteu method.[20] To 40 µL of extract, 0.5 mL of the Folin–Ciocalteu reagent and 2 mL of 20% sodium carbonate (Na_2CO_3) were added, and distilled water to reach a total of 10 mL. The samples were incubated for 2 h in the dark and at room temperature. Absorbance was measured at 765 nm using a spectrophotometer. The total phenolic content was calculated from the calibration curve and the results were expressed as µg of gallic acid equivalent per gram of dry extract (mg equivalent of GAE/g dry extract).

Antioxidant Activity Determination: The antioxidant activity was studied by evaluating the free radical-scavenging effect using DPPH (2,2-Difenil-1-picrilhidrazilo).[19],[21] A 500 µL of zucchini methanolic extract was mixed with 500 µL of ethanol and 125 µL of DPPH (0.02%). The mixture was thoroughly vortex-mixed and kept in the dark 60 min. The absorbance was measured at 517 nm using a UV-Visible spectrophotometer (UV-2550, Shimadzu, Kyoto, Japan) against a blank of methanol without DPPH. Results were expressed as the percentage inhibition of the DPPH radical, which was calculated using the equation:

$$\%\text{ inhibition of DPPH} = \left(\frac{Abs\ CONTROL - Abs\ SAMPLE}{Abs\ CONTROL}\right)\text{x}100$$

where *Abs CONTROL* is the absorbance of DPPH with methanol, and *Abs SAMPLE* is the absorbance of the DPPH with the zucchini methanolic extract. Then, the antioxidant activity was calculated as ascorbic acid equivalent antioxidant capacity (AAE, mg ascorbic acid/g dry extract).

Instrumental texture analysis

The samples were analyzed using the Texture Analyzer (TAXT2, Stable Micro System, UK) with an activity charge of 0.05 N. A cutting or shearing test was carried out using the model HDP/KS5 probe and with the Kramer Shear Cell with five blades. The analysis was done with a distance of 50 mm and at a speed of 1 mm/s. The attributes hardness (g), and adhesiveness (mJ) were recorded.

Sensory analysis

The sensory analysis was carried out in line with international standards. The tests were developed in a standard room equipped with 10 individual tasting areas.[22] The tasting panel, made up of 25 nonspecialist people were given a questionnaire together with cooked samples of which each was coded with two random numbers. These samples were served at room temperature and displayed on glass plates. Mineral water was provided to clean their palates after evaluating each sample.[23] An acceptance test was used applying a hedonic scale structured in 5 points, 1 being I dislike it a lot and 5 being I like it a lot to evaluate the characteristics of appearance, color, odor, taste, texture, hardness, adhesiveness, and general acceptation.

Statistical analysis

All of the determinations, with the exception of the sensorial analysis, were carried out in triplicate and the results expressed with the mean and standard deviation. Analysis of variance using a two-way ANOVA procedure and Tukey´s test were performed to determine the effect of treatment (fresh or freezing) in the nutrition composition, bioactive compounds, and sensorial analyses of the zucchini. The statistical differences were given when $p < .05$. Statistical analysis was carried out using the SPSS version 21.0 software package (IBM Corporation, Armonk, NY, USA).

Results and discussion

Nutritional and chemical profile

The results presented in Table 1 analyze the study of the influence of freezing and type of culinary treatment on the physicochemical characteristics of zucchini. Freezing and cooking were found to significantly affect the zucchini's moisture, protein, and fat. Ash was only modified by the effect of cooking. Freezing did not produce an appreciable loss of moisture in uncooked zucchini, compared to fresh, with moisture values being 95.21% and 95.34%, respectively. Steaming also did not cause an important decrease in the moisture of fresh (95.03%) or frozen zucchini (94.52%), although slightly lower values could be observed in the latter. Stir-frying did cause a significant loss of moisture in the zucchini, being greater in those that had previously been frozen, probably due to the breakdown of vegetable tissues that promotes even more water release after heat treatment.[24]

The moisture decreases as the temperatures applied in the heat treatment are higher justifying that stir-fried zucchini, whether fresh or frozen, presents lower moisture than that of steam cooking.[25]

Table 1. Changes in nutrition composition of fresh and frozen zucchini during cooking (g/100g of zucchini).

Industrial treatment	Culinary treatment		Moisture	Ash	Protein	Fat
Fresh	Raw		95.34 ± 0.19 ^{d}	0.32 ± 0.11 ^{a}	1.51 ± 0.07 ^{a}	0.08 ± 0.0 ^{a}
	Stir-frying		91.80 ± 0.26 ^{b}	0.82 ± 0.15 ^{b}	2.08 ± 0.17 ^{b}	0.44 ± 0.16 ^{b}
	Steam		95.03 ± 0.22 ^{cd}	0.55 ± 0.01 ^{a}	1.34 ± 0.03 ^{a}	0.09 ± 0.06 ^{a}
Frozen	Raw		95.21 ± 0.08 ^{cd}	0.47 ± 0.01 ^{a}	1.47 ± 0.04 ^{a}	0.09 ± 0.02 ^{a}
	Stir-frying		88.88 ± 0.53 ^{a}	1.02 ± 0.06 ^{b}	2.70 ± 0.08 ^{c}	0.98 ± 0.06 ^{c}
	Steam		94.52 ± 0.09 ^{c}	0.49 ± 0.01 ^{a}	1.47 ± 0.06 ^{a}	0.07 ± 0.02 ^{a}
Results of ANOVA		Freezing	0.000	0.147	0.002	0.038
	P-value	Cooking	0.000	0.000	0.000	0.000
		Interaction	0.000	0.227	0.002	0.000

Values are mean ± SD (n=3).
$^{a-d}$Different letter in the same column indicate a significant difference (p ≤ 0.05) between the samples.

The content of ash, protein, and fat was significantly higher in stir-fried zucchini, whether in fresh (0.82%, 2.08%, and 0.44%, respectively) or frozen (1.02%, 2.70%, and 0.98%, respectively), than in raw and steam cooked, possibly owing to the concentration of substances as a result of moisture loss.

In the case of ash, it could add view of the fact that no water is used during the stir-frying process, which implies a minor loss of water-soluble components. The increase in fat concentration could be due to the evaporation of water from the surface layers of the zucchini during the stir-frying stage since the oil used is a heat transfer medium.[6,26,27] In fact, it has been observed that the structural changes that a food undergoes during the freezing process favor the subsequent absorption of oil.[28]

Antioxidant properties

The results obtained in the determination of phenols content and antioxidant activity of zucchini are shown in Table 2. Freezing significantly affected both phenol content and antioxidant activity. In frozen raw zucchini (0.12 µg GAE/g dry extract) a decrease in the content of phenolic compounds is observed with respect to fresh samples (0.15 µg GAE/g dry extract), coinciding with the results obtained in a study of the effect of freezing on phenolic content in potatoes.[29] The consumption of raw potatoes provides higher levels of phenolic compounds to the diet, than if consumed after freezing.[30] On the other hand, stir-fried frozen zucchini had a higher phenolic content (0.14 µg GAE/g dry extract) compared to steamed frozen zucchini (0.12 µg GAE/g dry extract) and raw (0.12 µg GAE/g dry extract), probably as a result of a gain in phenols content from the oil used to stir-fry the zucchini. Similar conclusions have been reached in other studies on aubergine and potato chips.[31] The reduction of phenol concentration in steam-cooked zucchini is caused by making soluble a part of the phenols present in the food, provoking the destruction of cell walls and subcellular compartments that increases the leaching of substances into the extracellular space.[32]

There was an appreciable decrease in antioxidant activity (Table 2) in raw zucchini after being frozen, going from 13.96 to 5.58 mg AAE/g dry extract. This decrease was more intense when zucchini was fried, going from 21.76 to 12.99 mg AAE/g dry extract, or steaming, going from 16.20 to 5.61 mg AAE/g dry extract. The decrease in total phenols and antioxidant activity in frozen zucchini could be a result of the heat treatment which produces a softening of the vegetable tissue, this being more notable during stir-frying process, since higher temperatures are applied, thus facilitating the extraction of phenolic compounds of the cell matrix.

Phenolic compounds are soluble in water; therefore, culinary techniques using this medium, such as steam cooking, lead to a loss of phenols in the food.[33] These findings have led to the research in this paper. On the other hand, the increase in the antioxidant activity of zucchini during the stir-frying stage may be due to the use of extra virgin olive oil, since it can provide the food with its own

Table 2. Changes to the bioactive compounds of fresh and frozen zucchini during cooking.

Industrial Treatment	Culinary Treatment		Total Phenolic (µg GAE/g dry extract)	Antioxidant Capacity (mg AAE/g dry extract)
Fresh	Raw		0.15 ± 0.01 c	13.96 ± 0.70 b
	Stir-frying		0.15 ± 0.01 c	21.76 ± 2.68 c
	Steam		0.14 ± 0.01 bc	16.20 ± 0.38 b
Frozen	Raw		0.12 ± 0.00 a	5.58 ± 1.31 a
	Stir-frying		0.14 ± 0.00 bc	12.99 ± 1.59 b
	Steam		0.12 ± 0.00 ab	5.61 ± 1.24 a
Results of ANOVA		Freezing	0.001	0.000
	P-value	Cooking	0.224	0.000
		Interaction	0.149	0.742

Values are mean ± SD (n=3).
a-cDifferent letter in the same column indicate a significant difference (p ≤ 0.05) between the samples.
µg GAE/ g dry extract: microgram of gallic acid equivalent per gram of g dry extract.
mg AAE/g dry extract: milligram of ascorbic acid equivalent per gram of g dry extract.

antioxidants such as tocopherol, squalene, and avenasterol, causing an enrichment of the vegetable with this vitamin, resulting in the high values regarding steam cooking.[6] The antioxidant activity decrease in vegetables that have been processed,[34] verifying the lower values found in frozen zucchini than in fresh, as observed in this study (Table 2). On the other hand, factors related to cooking (culinary treatment used, temperature and time) have been shown to strongly affect the antioxidant activity of food.[35]

Instrumental texture profile analysis (TPA)

The results of texture (hardness and adhesiveness) for the zucchini are illustrated in Table 3. Freezing caused a significant decrease in hardness and adhesiveness in raw zucchini. This may be due to the formation of large ice crystals, of the extracellular and intracellular water of the plant, leading to the breakage of its cell walls.[15] It can also be caused by pre-blanching treatment, which is carried out during industrial freezing, leading to the solubilization and depolymerization of polysaccharides, which consequently affects the hardness of vegetables. In this sense, found that the longer the blanching time of peppers, green beans, aubergine, and beets (85–90ºC) the lower the firmness value in all the vegetables studied,[36] coinciding with the values obtained in cabbage.[37]

The culinary treatment affected the hardness and adhesiveness differently in fresh and frozen zucchini. In its fresh state, a significant decrease in hardness and adhesiveness is observed, both when steamed and stir-fried. In the case of hardness, significantly lower values are observed in steamed zucchini. These changes are related to thermal treatment causing the degradation of pectin and hemicellulose that make up the cell walls of vegetables.[38] However, in previously frozen zucchini, no significant differences were observed after culinary treatment, perhaps since the freezing process has already modified the texture as previously explained.

Sensorial characteristics

Table 4 shows the results, obtained by a consumer panel, about the effect of freezing and culinary treatment on the acceptance of the sensory characteristics of zucchini. The frozen zucchini obtained significantly lower values in all the sensory attributes, compared to the fresh zucchini, in both culinary treatments. This causes the general acceptance of frozen zucchini to be lower than that of fresh zucchini. In general, fresh-steamed zucchini is better valued than stir-fried with statistically significant differences in appearance, color, texture, and general acceptance. In frozen zucchini, a slightly higher acceptance was observed in most of the attributes in stir-fried, although in no case was it significant. It should be noted that the acceptance of the adhesiveness and hardness of fresh zucchini was greater than that of frozen zucchini, since no

Table 3. Changes in the texture of fresh and frozen zucchini during cooking.

Industrial Treatment	Culinary Treatment		Hardness (g)	Adhesiveness (mJ)
Fresh	Raw		50900 ± 2800 c	86.37 ± 46.73 b
	Stir-frying		18800 ± 14800 b	39.03 ± 2.66 a
	Steam		12000 ± 504.59 a	41.87 ± 4.39 a
Frozen	Raw		18000 ± 1200 b	11.40 ± 1.74 a
	Stir-frying		18100 ± 2571.42 b	34.37 ± 4.76 a
	Steam		18500 ± 1474.48 b	34.65 ± 8.47 a
Results of ANOVA		Freezing	0.000	0.000
	P-value	Cooking	0.000	0.000
		Interaction	0.000	0.000

Values are mean ± SD (n=3).
a-cDifferent letter in the same column indicate a significant difference ($p \leq 0.05$) between the samples.
g: grams.
mJ: miliJoules

Table 4. Changes in the sensory attributes of fresh and frozen zucchini during cooking.

Industrial Treatment	Culinary Treatment		Appearance	Colour	Odour	Taste
Fresh	Stir-frying		3.46 ± 0.18 [b]	3.56 ± 0.17 [b]	3.28 ± 0.16 [b]	3.18 ± 0.17 [b]
	Steam		3.98 ± 0.13 [c]	4.06 ± 0.11 [c]	3.44 ± 0.12 [b]	3.24 ± 0.16 [b]
Frozen	Stir-frying		2.70 ± 0.15 [a]	2.80 ± 0.18 [a]	2.90 ± 0.12 [a]	2.68 ± 0.16 [a]
	Steam		2.42 ± 0.13 [a]	2.38 ± 0.14 [a]	2.66 ± 0.13 [a]	2.28 ± 0.13 [a]
Results of ANOVA		Freezing	0.000	0.000	0.000	0.000
	P-value	Cooking	0.426	0.796	0.760	0.270
		Interaction	0.009	0.003	0.128	0.136
Industrial Treatment	Culinary Treatment		Texture	Hardness	Adhesiveness	G. Acceptation
Fresh	Stir-frying		3.38 ± 0.15 [b]	3.50 ± 0.13 [b]	3.16 ± 0.14 [ab]	3.34 ± 0.14 [b]
	Steam		3.82 ± 0.13 [c]	3.60 ± 0.13 [b]	3.40 ± 0.15 [b]	3.50 ± 0.12 [b]
Frozen	Stir-frying		2.98 ± 0.15 [a]	2.92 ± 0.14 [a]	2.90 ± 0.12 [a]	2.80 ± 0.15 [a]
	Steam		2.94 ± 0.13 [a]	2.82 ± 0.15 [a]	2.90 ± 0.14 [a]	2.48 ± 0.12 [a]
Results of ANOVA		Freezing	0.000	0.000	0.006	0.000
	P-value	Cooking	0.151	0.000	0.386	0.553
		Interaction	0.085	0.472	0.386	0.076

Values are mean ± SD (n=3).
[a-c]Different letter in the same column indicate a significant difference ($p \leq 0.05$) between the samples.

significant differences were observed at an instrumental level in these parameters, with the exception of fresh-steamed zucchini.

Conclusion

The freezing process did not significantly affect the nutritional composition of raw zucchini; however, a loss of phenolic compounds and antioxidant activity was observed in addition to a decrease in hardness and adhesiveness. It also causes a change in zucchini after cooking, such as decrease in phenolic compounds and antioxidant activity, as well as higher moisture losses and increased fat, ash, and protein in stir-fried. The steam cooking modified less the nutritional characteristics of the raw zucchini but did not provide greater antioxidant capacity or phenol content. There were no significant differences between the sensory acceptance of steamed and stir-fried zucchini. Stir-frying improved the healthy properties of zucchini (antioxidant capacity and phenolic compounds), and although it caused a slight increase in the caloric value, due to the loss of moisture and the increase of unsaturated fats from olive oil, globally considered to be the most suitable method for cooking zucchini.

Funding

This research was financially supported by Instituto de Fomento de la Región de Murcia (INFO) through project: “Design and development of culinary treatments for frozen vegetables to obtain a high nutritional and sensory quality.”

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