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Ready-to-Use Vegetable Salads: Physicochemical and Microbiological Evaluation

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Abstract: Ready-to-use vegetable salads are minimally processed products, rich in antioxidants, but are associated with a high microbiological risk and possibly, in some cases, with a high content of nitrites. The purpose of this study was to investigate the physicochemical and microbiological properties of different ready-to-use vegetable salad assortments on the Romanian market. Seventeen types of salad vegetables were evaluated for the determination of water activity, antioxidant activity and nitrite concentration and tested for the presence of microorganisms. The water activity of the samples varied from 0.873 to 0.933, and the IC50 values were between 1.31 ± 0.02 and 5.43 ± 0.04 µg/mL. Nitrites were present in all samples investigated (ranging from 290.6 to 3041.17 mg/kg). *Staphylococci* and *Enterobacteriaceae* were detected in 35.3% and 70.5% of the samples. Furthermore, 17.6% of the salads were contaminated with *Escherichia coli*, and *Listeria* was detected in 29.4% of the samples. *Salmonella* was detected in only one sample, and *Faecal streptococci* were not present in any of the samples. The results indicated high nitrite values and also revealed pathogens' presence. Producers should make more efforts to lower microbial contamination, while maximum limits for nitrites in vegetables should be set based on the impact on human health.

Keywords: DPPH; *Listeria*; ready-to-use; nitrites; *Salmonella*; vegetable salads

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1. Introduction

People's eating habits are influenced by the population's lifestyle, and it seems that nowadays, life is much more hectic with people having less free time; therefore, ready-to-use products are more and more preferred [1,2]. Ready-to-use products do not require additional preparation before being consumed and can be found on the market in a wide variety, like fresh-cut fruits, salads and vegetables, cooked meat and poultry, smoked/salted seafood, smoked/salted meat, and dairy [1]. Ready-to-use vegetable salads are minimally processed products [3], by sorting, cutting, washing/disinfection, rinsing, centrifugation, packaging in air or a modified atmosphere, refrigeration and transport, which are finally consumed in a short period of time [4,5]. According to Ülger et al. [6], about 10,000 plant species are considered vegetables, and they are classified as follows: leaf vegetables (chard, chicory, curly lettuce, lettuce, purslane and spinach); stalk vegetables (celery and asparagus), flowering vegetables (broccoli, cauliflower and artichoke); root, bulb and tuber vegetables (beet, carrot, fennel, onion, potato, radish and turnip); and legumes (peas and soya beans). In 2022, 59.8 million tons of vegetables were produced in the EU, with Spain as the leading producer, followed by Italy [7]. The highest number of farms growing fresh vegetables was in Romania, but the average area is much smaller than the EU average (7000 m^2 compared with 27,000 m^2). In 2020, 225.4 thousand ha of vegetables were cultivated in the country, with a total production of 3501.4 thousand tones (cabbage, with 977.4 thousand tones, and tomatoes, with 712.2 thousand tones, being the most cultivated). Rich in vitamins, minerals, dietary fiber content and phytochemicals, vegetables play an important role in human health [5], and it is recommended to consume 400 g of vegetables and fruits per day (of which 240 g should be vegetables) [8,9] for the prevention of stroke, high blood pressure, cardiovascular diseases and other micronutrient deficiencies [10]. In 2021, an average of 364.58 g of fruit and vegetables per day per capita were consumed in the EU, being below the minimum recommended [11]. Vegetables are consumed less in countries like Romania, Latvia and Bulgaria [12]. In 2019, only 2% of the Romanian population consumed five portions of fruits and vegetables per day [13] due to their income, habits and preferences, such as the choice to buy fresh, locally produced vegetables that are in season [14]. Increasing consumption of antioxidant-rich vegetables (carrots are rich in α-carotene and β-carotene; cabbage in ascorbic acid; lettuce and kale in vitamin E) will contribute to human health and well-being [10]. On the other hand, vegetable consumption may be associated with an increased intake of nitrites [15] present in vegetables due to mineral fertilizers applied to the soil [16]. The WHO has fixed an acceptable daily intake (ADI) of 0.07 mg nitrite/kg body weight based on methemoglobinemia risk data [17]. Ready-to-use vegetable salads are linked with a high microbiological risk because they are minimally processed products [18]. Microbiological contamination occurs due to microorganisms being present in the soil and water used for irrigation, as well as due to processing, transport and storage conditions [18]. According to Mir et al. [4], various microbial pathogens, including *Escherichia coli*, coliforms, *Salmonella*, and *Listeria*, as well as total aerobic bacteria and spoilage bacteria, yeasts and fungi, may be present in ready-to-use vegetable salads at high levels. *Escherichia coli* and *Salmonella* spp. cause symptoms of gastroenteritis (every year, 550 million people suffer from *Salmonella* poisoning [3]), while *Listeria monocytogenes* causes listeriosis [19] (1 million people suffer from listeriosis per year, and the number of poisonings is constantly increasing [3]). In the EU, EC Regulation 1441/2007 stipulates that concentrations of *Listeria monocytogenes* must be less than 100 colony-forming units (CFU)/g and *Salmonella* spp. must be absent, while the limit for *E. coli* bacteria is 1000 CFU/g vegetables. There are no mandatory microbiological criteria that include an assessment of total aerobic mesophiles, coliforms, yeasts and molds, but high levels could be an indicator of inadequate processing [18].

The aim of this study was to provide information on antioxidant activity, to determine nitrite concentrations and to evaluate the microbiological quality of ready-to-use vegetable salads available on the Romanian market. Also, this research aimed to determine the correlation among the physical–chemical (water activity, antioxidant activity, nutritional value and nitrites) and microbiological (total number of germs, yeasts and molds, as well as faecal streptococci, *Staphylococcus*, *Listeria*, *Enterobacteria*, *Salmonella*, *Escherichia coli* and total coliforms) parameters of ready-to-use vegetable salads.

2. Materials and Methods

2.1. Samples

Seventeen types of ready-to-use vegetable salads were purchased (March–April 2023) from four different chain stores in Suceava, Romania. Salads, mainly containing leafy vegetables that were available in the supermarkets at the time, were considered. The countries of the salad's origin were Italy and Romania, and their weight was 160, 200 and 260 g. The salads chosen were those only with ingredients of vegetable origin that were packaged and would be consumed without any cooking, further washing or preparation by the consumer. Spicy or seasoned salads were specifically excluded from the study. The products were purchased before the expiry date, were fresh and were placed in refrigerated boxes and transported to the university's laboratory. All samples were stored at a temperature of 4–8 °C and were tested on the purchase day. Table 1 contains the details of the ingredients found in ready-to-use salad bags, together with a specific coding for each variety.

Table 1. Ingredients of the ready-to-use vegetable salad assortments.

Ready-to-Use Salad Assortment	Coding	Ingredients
Bacio Salad	1L	Garden chicory, red beetroot, escarole;
Amorino Salad	2L	Pan di Zucchero chicory, escarole, red-leaved chicory;
Fresco Salad	3L	Curly garden chicory, red cabbage, red leaf chicory, corn kernels;
Silhouette Salad	4L	Curled-leaved endive, "Golden Heart" chicory, rocket, red-leaved chicory;
Lupino Salad	5L	Pan di Zucchero chicory, white cabbage, carrot, red-leaved chicory;
Rhapsody Salad	1K	Iceberg lettuce, romaine, radicchio, carrot, creamy endive;
Dacia Salad	2K	White cabbage, carrot, endive lettuce;
Joy Salad	3K	Red cabbage, endive lettuce, carrot;
Fantasy Salad	4K	Endive lettuce, curly endive, radicchio, spinach;
Garden Salad	5K	Romaine lettuce, leek, radish;
Energy Salad	1A	Curly endive, curly endive "heart of gold", red beetroot, pumpkin seeds, sunflower seeds;
Supreme Salad	2A	Iceberg lettuce, carrot, red cabbage, celeriac;
Flavor Salad	3A	"Lollo rossa" lettuce, "Oak leaf" lettuce, "Bull's blood" beetroot leaves, lamb's lettuce;
Pastel Salad	4A	Whole leaf garden chicory, "Pan di Zucchero" garden chicory, Curly garden chicory, red leaf chicory, round carrot, round red radish;
Lettuce Bowl Salad	1P	"Oak leaf" lettuce;
Sweet Crisp Salad	2P	Whole leaf chicory, "Pan di Zucchero" chicory, curly chicory, red-leaved chicory, carrot;
Mixed Salad	3P	"Pan di Zucchero" chicory, carrot, red-leaved chicory, arugula, lamb's lettuce;

The nutrition values of the ready-to-use salad assortments according to the manufacturer are given in Table 2.

Table 2. Nutritional value in 100 g of ready-to-use vegetable salad products (according to label of producers).

Ready-to-Use Salad Assortment	Energy Value (kJ/kcal)	Fat (g)	Saturated Fatty Acids (g)	Carbohydrates (g)	Sugars (g)	Fiber (g)	Protein (g)	Salt (g)
1L	80/19	0.2	0.0	1.9	1.1	2.5	1.2	0.15
2L	75/19	0.2	0.1	2.6	1.6	0.6	1.1	0.02
3L	132/23	0.7	0.2	3.5	2.0	2.4	1.6	0.15
4L	78/19	0.3	0.1	2.0	0.5	0.5	2.0	0.03
5L	88/21	0.2	0.1	2.9	0.9	1.8	1.0	0.05
1K	64.8/15.49	0.22	0.0	4.29	2.2	-	1.1	0.02
2K	106.4/25.43	0.28	0.0	6.98	4.4	-	1.2	0.06
3K	106.4/25.43	0.28	0.0	6.98	4.4	-	1.2	0.06
4K	60/14.3	0.20	0.0	2.80	1.3	-	1.8	0.09
5K	58.2/13.9	0.20	0.0	3.80	1.6	-	1.2	0.02
1A	237/57	3.41	0.5	2.50	0.1	1.8	3.1	0.26
2A	77/18	0.10	0.1	1.52	0.2	2.5	0.9	0.08
3A	47/11	0.18	0.1	0.50	0.1	1.6	1.6	0.17
4A	56/14	0.10	0.1	1.36	0.1	2.2	0.9	0.10
1P	45/11	0.0	0.0	2.10	0.0	-	1.4	0.03
2P	86/20	0.20	0.1	3.60	1.1	-	1.0	0.20
3P	124/29	0.0	0.0	5.20	1.4	-	2.1	0.10

2.2. Chemicals, Working Standard Solutions and Culture Media

All chemicals and reagents (2-diphenyl-1-picrylhydrazyl, methanol, $HgCl_2$ solution, sulphanilic acid and α-naphthylamine) used in the present study in the physicochemical experiments were purchased from Sigma-Aldrich (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany).

Culture media like Nutrient Agar, Malt Extract Agar, Bile Aesculin Azide Agar, 3M Petrifilm Staph Express discs containing O-toluidine blue and deoxyribonucleic acid (DNA), 3M Petrifilm Environmental *Listeria* Plates, XLD Agar medium, 3M Petrifilm *Enterobacteri-*

aceae Count Plates and 3M Petrifilm Rapid *E. coli*/coliform Count plates were purchased from Merck (Darmstadt, Germany).

2.3. Physicochemical Analysis

The water activity (aw) of ready-to-use vegetable salad samples was measured by using a water activity meter, AquaLab Lite (Decagon, WA, USA) [20].

2-Diphenyl-1-picrylhydrazyl (DPPH) Assay: The antioxidant activity of the samples was determined using the DPPH assay [21]. A total of 2.5 g of the test sample (ready-to-use salad) was weighed and then transferred to a 50 mL volumetric flask and made to the mark with 99.8% methanol. The samples were shaken vigorously for 5 min (liquid samples). After shaking, the samples were filtered using filter paper, and the resulting liquid was kept in covered containers to prevent evaporation. Fresh DPPH solution was prepared before the determination as follows: concentration 25 mg (0.025 g/L, methanol/water = 50:50), 3 mL of the DPPH solution was pipetted into the plastic cuvette, and the absorbance was measured at the 515 nm wavelength [22–25] using a spectrophotometer (SHIMADZU UV-VIS 3600 Spectrophotometer, Duisburg, Germany). Then, a 5 µL sample was added to the cuvette, shaken gently and allowed to rest for 5 min. The half-maximal inhibitory concentration (IC50) was calculated according to Prisacaru et al. [26,27].

Nitrite determination: 10 g of the well-shredded and homogenized ready-to-use vegetable salad samples were weighed, which were brought with approx 80 mL of distilled water in a 100 mL volumetric flask. The balloon was kept in the water bath for approx 60 °C for one hour, shaking vigorously from time to time [28]. A total of 5 mL of saturated $HgCl_2$ solution was then added, vigorously homogenized, cooled, made up to the mark with water and filtered.

The detection of nitrite was performed according to the following method [29]: 1 mL of Griess reagent, 1 mL of aqueous extract from the sample and 8 mL of water were introduced into a clean test tube. After homogenization, it was left to rest at room temperature for at least 20 min for color development, after which it was measured at 525 nm on a spectrophotometer against a distilled water blank [29].

2.4. Microbiological Analysis

The microbiological load was analyzed for each ready-to-use vegetable salad, namely the total number of germs (TNGs), yeasts and molds, as well as *Faecal streptococci*, *Staphylococcus*, *Listeria*, *Enterobacteria*, *Salmonella*, *Escherichia coli* and total coliforms. Ready-to-use salad samples were processed under sterile conditions to obtain an extract by processing 10 g of product with 90 mL of sterile solvent. From the initial dilution (sample extract), a series of decimal dilutions were made in order to grow on the culture media for the counting and identification of microorganisms.

Standard SR EN ISO 4833-2/2014 [30] was considered for the determination of the TNGs. The total germ count was determined by culturing on Nutrient Agar culture medium; the plates were incubated at 35 ± 2 °C for 18–24 h. Funke Gerber ColonyStar (Funke Gerber, Berlin, Germany) was used for colony counting [31].

The yeasts and molds number was established by culturing on Malt Extract Agar culture medium with incubation at 30 ± 2 °C for 18–48 h.

The number of *Faecal streptococci* was determined by growing on the Bile Aesculin Azide agar culture medium, and the plates were cooled to 45–50 °C and poured into Petri dishes at a depth of 3 mm to 5 mm, according to SR EN ISO 7899-2:2002 [32].

Staphylococci numbers were determined by culturing on 3M petrifilm staph express discs containing O-toluidine blue and deoxyribonucleic acid (DNA), and the plates were incubated clear-side-up in stacks of no more than 20 plates.

Listeria counts were determined according to SR EN ISO 11290-1/2017 [33] by seeding on Petrifilm plates, and these were incubated clear-side-up in stacks of up to 10 for 28 ± 2 h at 35 °C ± 1 °C or 37 °C ± 1 °C. Funke Gerber ColonyStar (Funke Gerber, Berlin, Germany) was used for colony counting.

The number of *Enterobacteriaceae* was determined according to ISO 21528-2/2017 [34] by seeding on Petrifilm plates, and these were incubated clear-side-up in stacks of no more than 20 plates.

Salmonella was determined by culture on the XLD Agar medium, and the plates were incubated at 35 °C for 24–48 h according to ISO 6579-1:2017 [35].

Total coliform counts and *Escherichia coli* were determined according to ISO 4832/2009 [36] by seeding them on 3M Petrifilm rapid *E. coli*/coliform count plates and incubating them clear-side-up in stacks of no more than 20 plates.

2.5. Statistical Analysis

The analyses were carried out in triplicate, and the obtained results were reported as the mean with a standard deviation (mean ± SD). Minitab version 17 (State College, PA, USA) was used for the statistical analysis: ANOVA (95% confidence interval ($p < 0.05$)) with Tukey's test was considered to compare the physicochemical and microbiological results, and principal component analysis (PCA) and Pearson's correlation were conducted to determine the potential relationship between parameters.

3. Results and Discussion

The results of this study can be representative of the situations in other EU countries, especially in those where these types of salads are imported. The water activity (aw) of ready-to-use vegetable salad samples was between 0.873 and 0.933 (Figure 1), with values slightly lower than those reported by other studies. Zhang et al. [37] reported water activity being between 0.97 and 0.99 for ready-to-use vegetables, while Alegbeleye and Sant'Ana [38] obtained the aw as being between 0.95 and 0.99 for ready-to-use vegetable salads.

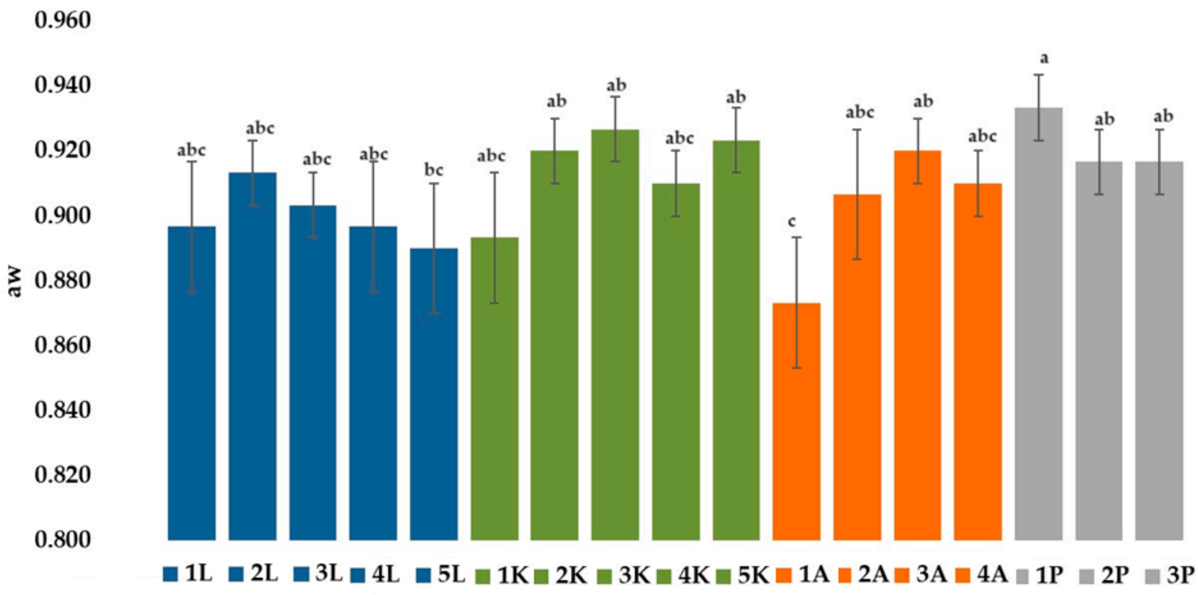


Figure 1. The aw of ready-to-use vegetable salads. Means with different lowercase letters (a–c) indicate significant differences ($p < 0.05$) among the samples.

In the present study, sample 1A, which contains curly endive, curly endive "heart of gold", red beetroot, pumpkin seeds, and sunflower seeds, had the lowest aw value (0.873 ± 0.03), while the highest value (0.933 ± 0.01) was registered for sample 1P, which included only "Oak leaf" lettuce. The addition of pumpkin and sunflower seeds probably led to a decrease in the aw value of the salad sample. According to Schmidt and Fontana [39], sunflower seeds can have a aw of 0.75, while dried pumpkin seeds can have a water activity of 0.486 [40]. Most of the ready-to-use vegetable salad samples investigated in this study did not have a significantly different value for the aw, according to Figure 1. Water activity significantly influences the growth of microorganisms [38], being a helpful indicator of the food products' microbiological stability [41]. According to Preetha and Narayanan [42], high moisture foods with a aw above 0.85 are highly perishable foods that are susceptible to increased spoilage and pathogenic microorganisms.

The DPPH results are illustrated in Figure 2 and are expressed as the inhibitory concentration at 50% (IC50). Small IC50 values indicate high antioxidant activity [27,43], while Thiangthum et al. [44] indicate a high antioxidant activity of samples when the IC50 is <30 μg/mL, an intermediate antioxidant activity when it is 30 < IC50 < 50 μg/mL, low when it is 50 < IC50 < 70 μg/mL and absent when the IC50 is >70 μg/mL. Figure 2 shows that the IC50 values are between 1.31 ± 0.02 μg/mL (sample 4L) and 5.43 ± 0.04 μg/mL (sample 1P), which means high antioxidant activity. Samples like 4L (curled-leaved endive, "Golden Heart" chicory, rocket and red-leaved chicory) and 1K (iceberg lettuce, romaine, radicchio, carrot and creamy endive) had higher scavenging activity of the extract, indicated by low IC50 values, while samples 1A (Curly endive, curly endive "heart of gold", red beetroot, pumpkin seeds and sunflower seeds) and 1P ("Oak leaf" lettuce) had lower scavenging activity of the extract.

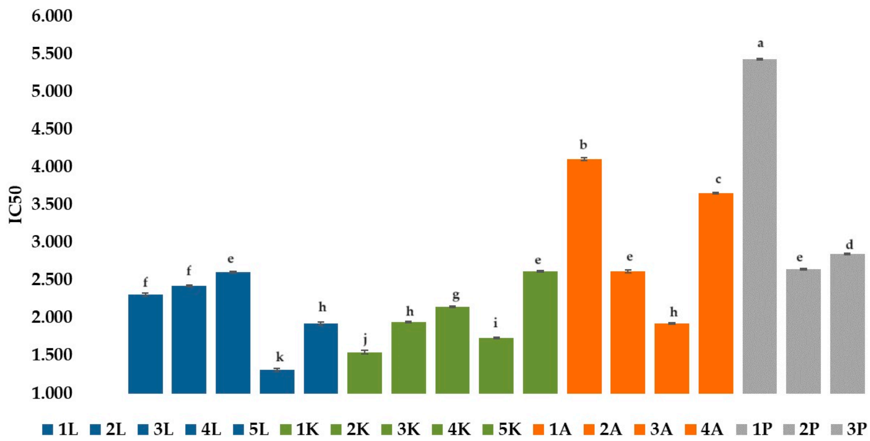


Figure 2. IC50 (μg/mL) of ready-to-use vegetable salads. Means with different lowercase letters (a–k) indicate the significant differences ($p < 0.05$) among the samples.

Nitrite was present in all investigated samples in the range of 290.6–3041.17 mg/kg (Figure 3).

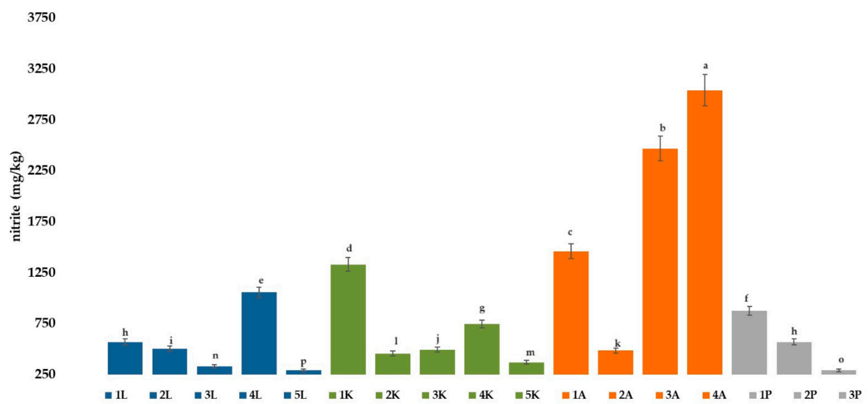


Figure 3. Nitrite of ready-to-use vegetable salads. Means with different lowercase letters (a–p) indicate the significant differences ($p < 0.05$) among the samples.

The lowest value was registered for sample 5L, which contains Pan di Zucchero chicory, white cabbage, carrot and red-leaved chicory, while the higher value was determined for sample 4A with the following ingredients: whole leaf garden chicory, "Pan di Zucchero" garden chicory, curly garden chicory, red leaf chicory, round carrot and round red radish. Leafy vegetables are high in nitrites; only "Oak leaf" lettuce (sample 1P) had 876.4 ± 0.04 mg/kg. High values for leafy vegetables (327.1 mg/kg for spinach, 378.1 mg/kg for lettuce and 537.9 mg/kg for rucola) were reported by Luetic et al. [16]. The high content of nitrites can be due to cultivation conditions (use of fertilizers containing nitrates and then the conversion of nitrates into nitrites under the action of microorganisms under certain conditions), processing (washing), packaging and storage. Nitrites are considered to be potentially harmful, but the concentration of nitrites in vegetables is not currently subject to any regulatory limitations [16]. The ADI for nitrites is between 0 and 0.07 mg/kg body weight/day, according to the European Food Safety Authority [45]. The ADI for nitrites corresponds to 4.2 mg for a person of 60 kg per day [16]; therefore, the ingestion of 100 g of "Oak leaf" lettuce (sample 1P) with a nitrite concentration of 876 mg/kg will result in an intake of 87.6 mg nitrite, which far exceeds the ADI. The results of this study indicated that for all investigated ready-to-use vegetable salad samples, the nitrite intake would far exceed the ADI. The impacts of nitrite (negative and positive) on human health are still under discussion. According to Cheng et al. [46], the clear cardiovascular benefits of dietary nitrite may outweigh the potential risks, but there are also studies connecting the consumption of nitrite-rich foods with diabetes [16].

Ready-to-use vegetable salad samples were characterized by different microbiological qualities. Figure 4 illustrates the presence of the total number of germs, *Staphylococci*, *Enterobacteriaceae*, *Salmonella* and total coliforms on different samples.

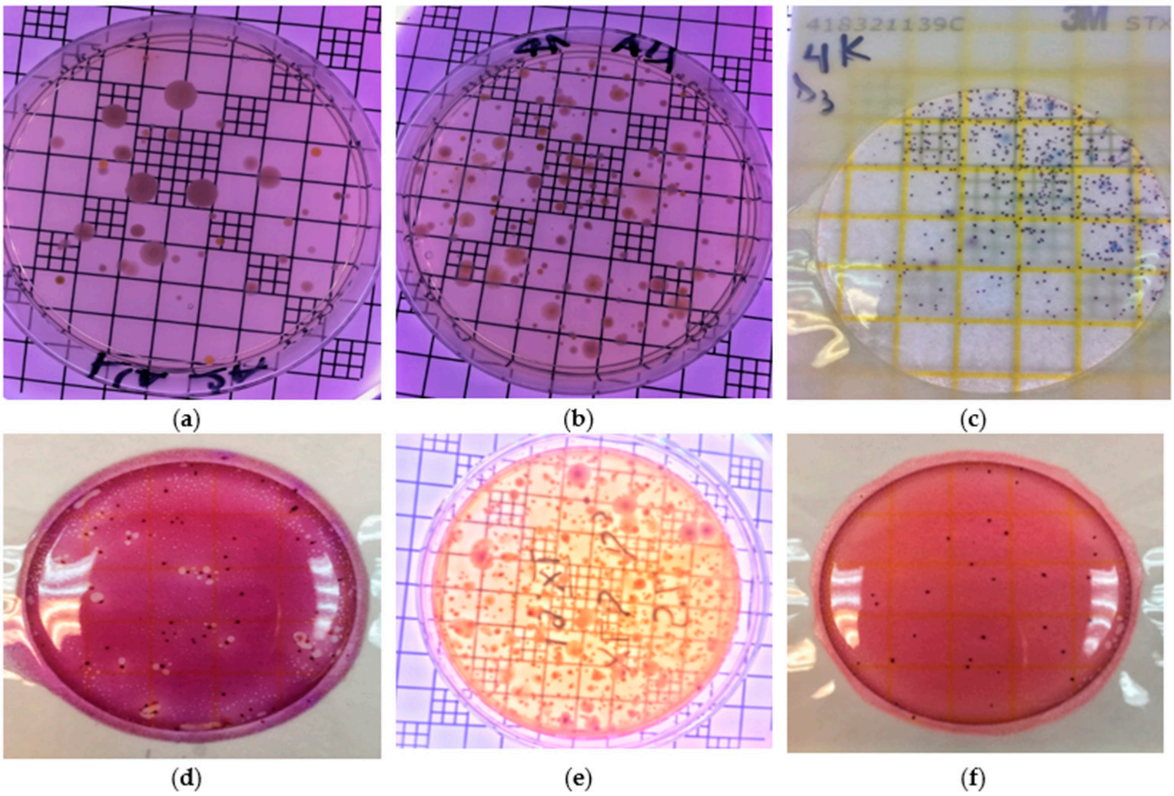


Figure 4. Total number of germs present on coded samples: (**a**) 2A; (**b**) 1P; (**c**) *Staphylococci* present on 4K-coded lettuce; (**d**) *Enterobacteriaceae* present on coded lettuce 3L; (**e**) *Salmonella* present on 5L sample; and (**f**) total coliforms present on 5L sample.

Table 3 presents the results of the microorganism number (total number of germs (TNGs), yeasts and molds (YM), *Staphylococci* (ST), *Enterobacteriaceae* (EB) and total coliforms (TC)).

Table 3. Microbial counts of the ready-to-use salads (in log CFU/g). Values with the different letters within one column are significantly different ($p < 0.05$).

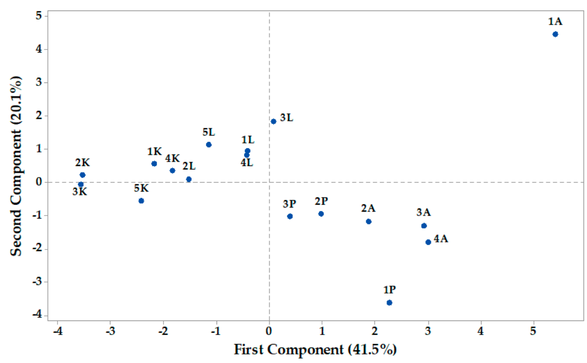
Sample	Total Number of Germs (TNGs)	Yeasts and Molds (YM)	*Staphylococci (ST)*	*Enterobacteriaceae* (EB)	Total Coliforms (TC)
1L	5.61 ± 0.02^{g}	4.56 ± 0.07^{a}	-	-	6.03 ± 0.02^{b}
2L	5.48 ± 0.06^{hi}	4.42 ± 0.10^{a}	-	-	6.12 ± 0.02^{b}
3L	5.42 ± 0.03^{i}	4.82 ± 0.04^{a}	-	5.28 ± 0.02^{d}	6.07 ± 0.02^{b}
4L	5.78 ± 0.01^{f}	2.67 ± 2.31^{a}	2.67 ± 2.31^{bc}	4.67 ± 0.06^{e}	6.09 ± 0.02^{b}
5L	5.54 ± 0.02^{gh}	5.48 ± 0.01^{a}	-	-	6.03 ± 0.02^{b}
1K	5.46 ± 0.01^{hi}	4.94 ± 0.03^{a}	1.33 ± 2.31^{c}	4.50 ± 0.17^{e}	6.39 ± 0.01^{a}
2K	5.56 ± 0.01^{gh}	4.80 ± 0.04^{a}	4.1 ± 0.17^{ab}	4.75 ± 0.08^{e}	6.37 ± 0.01^{a}
3K	5.50 ± 0.03^{hi}	4.00 ± 0.00^{a}	4.97 ± 1.29^{ab}	4.16 ± 0.28^{f}	6.33 ± 0.02^{a}
4K	5.62 ± 0.02^{g}	2.77 ± 2.40^{a}	5.97 ± 0.80^{a}	-	6.01 ± 0.02^{b}
5K	5.80 ± 0.00^{f}	5.17 ± 0.02^{a}	3.32 ± 2.87^{b}	-	6.01 ± 0.02^{b}
1A	7.47 ± 0.03^{d}	-	-	6.56 ± 0.03^{a}	5.66 ± 0.10^{de}
2A	7.59 ± 0.03^{c}	-	-	6.35 ± 0.06^{ab}	5.52 ± 0.07^{e}
3A	8.12 ± 0.01^{a}	-	-	6.04 ± 0.04^{c}	5.46 ± 0.15^{e}
4A	7.88 ± 0.03^{b}	-	-	6.40 ± 0.09^{ab}	5.50 ± 0.17^{e}
1P	8.09 ± 0.04^{a}	-	-	6.26 ± 0.01^{bc}	5.00 ± 0.00^{f}
2P	7.17 ± 0.09^{e}	-	-	6.20 ± 0.10^{bc}	5.75 ± 0.05^{cd}
3P	7.83 ± 0.04^{b}	-	-	6.33 ± 0.03^{ab}	5.94 ± 0.03^{bc}

There are no microbiological criteria set for ready-to-use foods in the EU. Only Commission Regulation 1441/2007, formerly Commission Regulation 2073/2005, is applicable [47,48] for pre-cut fruit and vegetables (ready-to-use): for *Salmonella*, an absence in 25 g, and for *E. coli*, 1000 CFU/g. The total viable count of microorganisms is not a legislative criterion for ready-to-use vegetable salads, but it is an important indicator of sensory and hygienic quality [3]. The TNGs ranged from 5.42 log CFU/g (sample 3L) to 8.12 log CFU/g (sample 3A), while yeasts and molds were mainly observed in groups L and K samples, with the lowest values for samples 4L (2.67 log CFU/g) and 4K (2.77 log CFU/g), and the highest values for samples 5L (5.48 log CFU/g) and 5K (5.17 log CFU/g) (Table 3).

The number of yeasts and molds was lower than the number of the TNGs. Also, the number of yeasts and molds are similar to those reported by Łepecka et al. [3] (1.0–7.0 log CFU/g ready-to-use salads), Jeddi et al. [49] (6.2–7.5 log CFU/g for ready-to-use salads and 5.4–7.6 log CFU/g for fresh-cut vegetables) and Badosa et al. [50] (5.0–7.0 log CFU/g for packed ready-to-use vegetables). *Staphylococci* were present only in the 4L sample (2.67 log CFU/g), and in the K group samples, they ranged from 1.33 log CFU/g (1K sample) to 5.97 log CFU/g (4K sample). High numbers of *Enterobacteriaceae* (EB) were found in samples from groups A (6.04–6.56 log CFU/g) and P (6.20–6.33 log CFU/g), which means a high degree of microbiological contamination (higher than 6 log CFU/g) [3], while total coliforms were present in all ready-to-use vegetable samples, with numbers between 5.00 log CFU/g (sample 1P) and 6.39 log CFU/g (sample 1K). Jeddi et al. [49] reported that the number of total coliform in fresh-cut vegetables and ready-to-use salads was 4.0 log CFU/g. No *Faecal streptococci* were present in any of the samples analyzed. *Listeria* was found only in samples 3L (2.67 log CFU/g), 4L (4 log CFU/g), 2L and 5K (4.20 log CFU/g), and 4K (4.36 log CFU/g) (5 out 17 ready-to-use vegetable salads (29.4%)), while *Salmonella* was detected only in sample 5L (4.90 log CFU/g) (1 out 17 ready-to-use vegetable salads (5.9%)). *Listeria* was detected by Badosa et al. [50] in 12 samples (ready-to-use vegetables like alfalfa sprout, spinach and mixed salad samples; carrot and curly endive), while *Salmonella* was detected in mixed salads. Jeddi et al. [49] reported that *Salmonella* was isolated from 1 out of 20 ready-to-use salad samples (5%). *Escherichia coli* was identified in three ready-to-use vegetable samples: 3L (4 log CFU/g), 5L and 1K (4.3 log CFU/g), all exceeding the allowed limit. In this study, *Escherichia coli* was detected in 3 out of 17 ready-to-use vegetable salads (17.6%), while Jeddi et al. [49] detected *Escherichia coli* in

6 out of 20 ready-to-use salad samples (30%). The contamination of vegetables and their degree of deterioration can be indicated by the number of microorganisms, which can also indicate the natural microbiota of the vegetables. Leafy vegetable cleaning is a crucial stage since they cannot be subjected to thermal treatment [3]. Control of microorganisms on leafy vegetables offers far fewer technologies than most other food categories because heat treatments, which are well-developed, are not feasible on vegetable leaves due to the perishability of the crop [51]. Physical methods of controlling microorganisms, in addition to heat treatment, include treatments such as washing, modified atmosphere packaging and radiation-based techniques. Methods such as immersion or running in water, the duration and number of washes, and the use of acetic acid, sodium bicarbonate and sodium chloride influence the vegetables' washing process and, implicitly, the removal of microorganisms and nitrite [16,52]. Pezzuto et al. [52] demonstrated that washing vegetables with sodium hypochlorite can result in a 2-log reduction in *Salmonella* counts, while a solution of sodium hypochlorite and combined peracetic and perchloric acids can significantly reduce *Listeria* counts. Ultraviolet light has been shown to be effective in reducing the microbial load on leafy lettuces (3-log reductions in the total viable count were obtained), although there is a possibility of leaf damage when exposure is excessive [53]. Chemicals, modified atmosphere packaging (CO_2 is the only gas with antimicrobial influence on food [54]), refrigeration, irradiation, high-pressure processing (200 and 400 MPa, 30 min at 5 °C results in a 2–4 log reduction in mesophilic bacteria, yeast and mold [55]), and essential oils (cinnamon leaf, oregano, rosemary, lemongrass and thyme [56]), and storage temperature (0–4 °C) may inhibit microorganism growth in the vegetables.

The relation between the physicochemical and microbiological parameters of ready-to-use vegetable salads is highlighted in Figure 5.



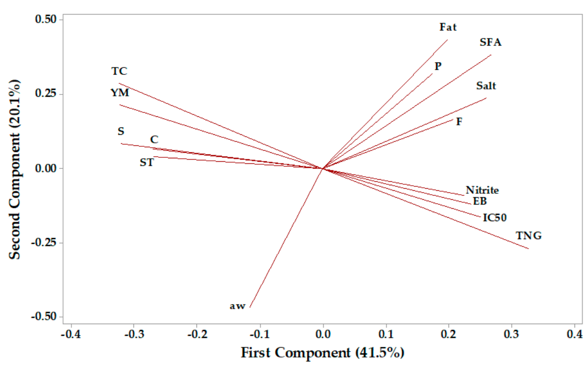


Figure 5. Principal component analysis (PCA) (**a**) scores and (**b**) loading plots of ready-to-use vegetable salad samples. Note: yeast and molds (YM), *Staphylococci* (ST), total number of germs (TNGs), *Enterobacteriaceae* (EB), total coliforms (TC), water activity (aw), half-maximal inhibitory concentration (IC50), saturated fatty acids (SFA), carbohydrates (C), sugars (S), fiber (F), and protein (P).

The principal component (PC1) had the highest eigenvalue of 6.23 (which explained 41.5% of the total variation), followed by PC2 with the eigenvalue of 3.01 (accounted for 20.1% of the total variation). Together, PC1 and PC2 accounted for 61.6% of the cumulative proportion of variance. Figure 5a shows that samples 1L, 2L, 4L, 5L, 1K, 2K, and 4K had negative scores for PC1 and positive scores for PC2, while 3K and 5K had negative scores for both PC1 and PC2. Samples 1P, 2P, 3P, 2A, 3A, and 4A had positive scores for PC1 and negative scores for PC2, while samples 1A and 3L had positive scores for both PC1 and PC2, according to Figure 5a. The yeast and molds number (−0.323), *Staphylococci* number (−0.269), the total coliforms number (−0.324), aw (−0.116), carbohydrates (−0.271) and sugars (−0.322) showed a negative correlation with PC1, while the other parameters were positively correlated with PC1 (the highest contribution had a TNGs with 0.328) (Figure 5b). PC2 showed a negative correlation with aw (−0.468), TNGs (−0.270), IC50 (−0.163), EB (−0.118) and nitrite (−0.091) and showed a positive correlation with the other investigated parameters (the highest contribution had fat, with 0.435). The analyzed parameters, such as TC, YM, S, C and ST, were grouped around the 1K, 2K, 4K, 1L, 2L, 4L and 5L samples, while nitrite, EB, IC50 and TNGs were more representative for the 1P, 2P, 3P, 2A, 3A and 4A samples, while fat, SFA, salt, P and F had higher values for the 1A and 3L samples.

Also, Pearson's correlation was used to investigate the obtained data, and the results showed positive correlations between the TNGs and nitrite (0.510) and EB and nitrite (0.403), and negative correlations between YM and nitrite (−0.473), TC and nitrite (−0.427) and ST and nitrite (−0.216).

4. Conclusions

In recent years, the consumption of fresh raw vegetables, especially in the form of pre-cut and ready-to-use salads, has increased worldwide. These products are minimally processed, which means that the risk of microorganism contamination is high. The results presented in this study confirm this aspect and indicate that there is a problem regarding the presence of pathogenic bacteria like *Listeria*, *Escherichia coli* and *Salmonella* in ready-to-use vegetable salads. Also, there were a high number of bacteria, yeast and molds in the samples. This study has shown that although the packaging of salads states that they are washed and ready to eat, in reality, some have a high microbial load, which poses a health risk. To prevent the growth of microorganisms on ready-to-use salads, good hygienic practices should be applied during growing and processing, from farm to fork. Water activity plays a very important role in the development of microorganisms, as many of them prefer water-rich food, which is a good environment for multiplication. Making a correlation between the water activity resulting in the samples analyzed and the activity of the microorganisms, it appears that in the sample coded 1P, the water activity is 0.93 at 23.9 °C, being the highest of all the samples analyzed, and the total number of germs is also the highest of all the samples analyzed (8.09 log CFU/g). The results indicate high nitrite levels (probably due to the use of fertilizers in high concentrations or other factors like atmospheric humidity, temperature and irradiance) in all samples, which greatly exceeds the acceptable daily intake. Due to the microbiological reduction in nitrates in vegetables, nitrite concentrations can increase. Therefore, bacterial growth can contribute to nitrite accumulation in ready-to-use vegetables.

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