Title

Microbiological characteristics and applied preservation method of novel ready-to-eat vegetarian spreads and dips

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Abstract

A range of spreads and dips as novel vegetarian ready-to-eat foods are being introduced on the (European) retail market to response to an increasing number of vegetarian'/flexitarian' consumers and the shift towards plant-based foods consumption. This innovative food group was explored by a product survey (n=369) in Belgium. The spreads and dips were classified on the presence of main ingredients, the applied preservation mode, the remaining shelf-life period, and the presence of additives. The majority of the products have soybeans or other seeds as the main ingredient, are containing preservatives (e.g. potassium sorbate; E202) and organic acids/acid regulators (e.g. lactic acid; E270 and citric acid; E330) as additives. 45% of the products were marketed under the organic label and 36 % of them were indicated as so-called 'clean label'. From a selection (78 refrigerated and 23 ambient stored products), the physicochemical characteristics (i.e. pH, water activity, and headspace gas composition) were determined as well as the microbiological quality, hygiene, and safety at the moment of purchase and the end of the indicated shelf-life. The analyzed microbiological quality indicators for refrigerated samples demonstrated a psychrotrophic total plate count range of 1.0-9.4 log CFU/g, psychrotrophic lactic acid bacteria range of 1.0-8.8 log CFU/g, yeast and fungi range of 2.0-9.5 log CFU/g and sulfite reducing *Clostridia* range of 1.0-3.7 log CFU/g. *E. coli* and coliforms were analyzed as hygiene indicators and solely coliforms were detected on three samples with a maximum count of 3.6 log CFU/g. *Listeria monocytogenes* was not detected in 10g. *Bacillus cereus* never exceeded 3.5 log CFU/g. For the ambient stored samples, mesophilic total plate count, mesophilic lactic acid bacteria, yeast and fungi, sulfite reducing *Clostridia*, *B. cereus, B. cereus* spores, aerobic spores, and anaerobic spores were determined. Most of the products showed cell counts below the limit of detection (= 1.0 log CFU/g). Only products based on chickpeas, sesame seeds, and other seeds showed cell counts for mesophilic total plate count and aerobic plate count ranging respectively from 1.0 to 4.3 log CFU/g and 1.0 to 5.1 log CFU/g. The research gives insight into the variable microbiological composition and multiple applied preservation modes of this innovative food group of ready-to-eat vegetarian dips and spreads.

Keywords

Vegetarian ready-to-eat foods, foods of non-animal origin, microbiological quality, additives, clean label

1. Introduction

Policymakers are convinced of the necessity to increase the consumption of plant-based products, taking into account sustainability needs. Lancet emphasized this in their EAT report and similar, the European Commission stated the necessity to shift towards a more plant-based diet in their Farm-to-Fork Strategy addressing a sustainable food system (EU Commission, 2020; Lancet Commission, 2019). Countries stimulate consumption of plant-based foods in their national dietary guidelines (FIA (food industry Asia) Communications, 2016; González-Fischer & Garnett, 2016; Lancet Commission, 2019; National institute of nutrition, 2014; Vlaams Instituut Gezond leven, 2017). Consumers are also more interested in plant-based products and an increase in consumption of convenient and ready-to-eat vegetarian spreads and dips (=VSD) is noticed e.g. an increase in the consumption of plant-based foods of 67% in Belgium was mentioned in the period of 2012-2018 (VMT-Food, 2019a). Food producers responded to this trend by extending and diversifying the offer in ready-to-eat vegetarian spreads and dips e.g. 239 new vegetarian spreads and dips were developed in the period 2011-2015 in Germany (Statista, 2016).

There is also an emerging trend among the (young) consumers who demand food that is more natural, organic, less processed, and contains fewer additives (Aschemann-Witzel et al., 2019; Asioli et al., 2017). A growing interest in ‘clean label’ products is noticed, in 2013 on average 78% of European consumers stated the list of ingredients as quite important, and accompanied by this, 27% of the newly released products in Europe were ‘clean label’ (Asioli et al., 2017; Ingredion, 2014). Many consumers perceived that additives are unhealthy and dangerous (Asioli et al., 2017) although the safety of every used additive in Europe is evaluated by the Scientific Committee on Food (SCF) and/or the European Food Safety Authority (EFSA) (EU Commission, n.d.). This poses hurdles for the food producers who strive to maintain the quality and safety of their products during the shelf-life period and so product formulation and preservation methods may need to be adapted.

Scientific research concerning the microbiological composition/stability during the shelf-life of this novel ready-to-eat product group of vegetarian spreads and dips is lacking. In past decades, the focus was made on the evaluation of the microbiological composition and stability of animal-based products such as dairy, meat, or fish and their derived foods-followed by fresh(cut) plant-based products such as ready-to-eat vegetables and salads because of their strong link with foodborne pathogens (EFSA, 2019).

The ready-to-eat vegetarian spreads and dips can pose a food safety concern as their production process doesn't necessarily include heat treatments or other microbiological reduction interventions (e.g. high-pressure processing). The FDA made 16 recalls for pesto, salsa, tapenade, and guacamole with mainly *Listeria monocytogenes*, *Clostridium botulinum,* and *Salmonella* spp. as the cause (FDA, 2019). The CDC reported 122 outbreaks concerning pesto, salsa, and guacamole between 1988 and 2017 (CDC, 2019). More recently, in 2019, 80 hummus products originating from one company in UK and Ireland were withdrawn in an extended recall from the European market due to the possible presence of *Salmonella* spp. (Food Standard Agency, 2019).

The increasing offer and consumption of convenient ready-to-eat spreads and dips and the trend towards the use of fewer additives create the necessity for research on the product formulation and preservation methods of this emerging food category. In this study, an elaborate product survey and a screening are presented to characterize this innovative product group of ready-to-eat vegetarian spreads and dips towards product composition (i.e. main ingredients), preservation method (i.e. physicochemical properties, presence of additives, and modified atmosphere packaging) and microbiological quality, hygiene and safety throughout the storage period to illustrate the broad range of current practices applied by the food industry.

2. Material and methods

The flow of the different steps in the research is represented in Figure 1.

2.1 Market study and categorization of the available vegetarian spreads and dips on the Belgian retail market

Between July and September 2018, retailers (i.e. Albert Heijn, Aldi, Bioase Natuurvoeding, Bioplanet, Carrefour, Colruyt, Delhaize, Lidle and Smatch) in Flanders, Belgium were visited to make an inventory for ready-to-eat prepacked vegetarian spreads and dips (VSD). The included foods could only contain plant-based main ingredients. An inventory database was created including the name of the product, manufacturer/retailer, list of ingredients (including additives), storage conditions (refrigerated or ambient stored), and expiration date. This information was retrieved from the label of the prepacked food or by consulting the online information of the retail shop or manufacturer. The database was used to make subcategories, based on different parameters as the main ingredient, additives, and, storage conditions. Dips and spreads containing a protein-rich main ingredient were assigned to one of the following subcategories: chickpeas, soybeans, legumes (excluding chickpeas and soybeans), sesame seeds, or other seeds. Products identified by a commercial name on the label were classified into one of the following commercial (recipe-linked) names: pesto, tapenade, salsa, or guacamole. The other products with a vegetable or herb as the main ingredient were assigned to the group 'other products with a vegetable or herb as base component'. Additives were retrieved from the list of ingredients of the prepacked products. The number of different applied E-numbers (= European additives) for each functional additive group was determined e.g. six different preservatives could be used in pesto. In case no additives were declared, the product was classified as 'clean label'. Also, the 'organic' label was added to this classification when the product was labeled as such (EC Regulation no. 834/2007).

2.2 Characterization of selected dips and spreads

From the listed products, 78 refrigerated products (in the same ratio as found in the market study to include the variability in the type of products) and 23 ambient stored products (two products for each subcategory except three for the pesto and four for the other spreads and dips with a vegetable or herb base) were selected to analyze physicochemical properties, microbiological composition, and the microbiological stability during the storage period (Figure 1). The refrigerated products were stored at 7 °C which is the upper bound of the recommended refrigerated storage temperature range in Belgium (FASFC, 2015). Fewer ambient stored samples were analyzed because it was expected that these were stable from a microbiological perspective. The analyzes started in September 2018 and were finalized in February 2019. At the moment of purchase, two or three (depending on the storage conditions) samples of a particular food product were bought with the same remaining shelf-life in the same retail shop, in order to have samples from a similar production batch. It is assumed that food producers are given a similar shelf life date to a certain production batch. Like this, the potential batch variability in further analyses is avoided.

2.2.1 Physicochemical characteristics of spreads and dips in relation to the microbiological growth potential of pathogens

The a_w (cryometer AWK-40-2, Pedak meettechniek BV) and pH (Seven Easy, Mettler Toledo / edge ® pH HI2002-02, Hanna Instruments) of 78 refrigerated products and 23 ambient were analyzed. The gas composition (gas analyzer type 302, Dansensor) of the headspace was measured for the refrigerated products.

2.2.2 Remaining shelf-life of vegetarian spread and dips

The remaining shelf-life was determined by the difference between the date of purchase and the indicated expiration date (‘use by’ or ‘best before’) on the package (Daelman et al., 2013). Different time intervals were attributed (less than 5 days, between 5 and 14 days, between 2 and 4 weeks, and more than 4 weeks) to categorize the products.

2.2.3 Microbiological composition of vegetarian spread and dips

The refrigerated samples were purchased in duplicate and analyzed on the day of purchase (=DP) and at the end of shelf-life (=ES) (Figure 1). Storage tests were conducted to determine the presence of different hygiene and quality indicators and their evolution over time. The ambient stored products were bought in triplicate and additionally, subjected to a forcing test. A first sample was analyzed at the DP, a second sample after a 7-day incubation period at 37 °C, and the third sample after a 7-day incubation period at 55 °C to determine the microbiological stability of products with a long shelf-life (AFNOR, 1997).

For each sampling 10 g of product was used to make the first dilution with PPS (peptone physiological solution, 8.5 g of salt, 1 g neutralized bacteriological peptone (LP0034, Oxoid) for 1 L water). Further dilutions with PPS were made if necessary. Several quality, hygiene, and safety indicators were determined to gain insight into the microbiological composition of VSD. In Table 1 the media, used supplements, incubation temperatures, incubation times, and used ISO methods of the streak plates are given. *Bacillus cereus* colonies were confirmed on blood agar plates. To determine the spores, 10 ml of the first dilution was heated to 80 °C for 10 minutes to eliminate the vegetative cells.

3. Results and discussion

3.1 Product study and categorization of the available vegetarian spreads and dips on the Belgian retail market

A total of 369 VSD were identified from retail stores in Flanders, Belgium, and their online catalogs in the period July – September 2018. The majority of the products are well-established vegetarian foods such as guacamole, pesto, salsa, and products based on chickpeas such as hummus. The list of ready-to-eat spreads and dips retrieved from the market study (Figure 2) is non-exhaustive and changes continuously due to adapting product formulations, innovation, and changing offer in the stores. Some items will remain popular year-round while others are seasonal and thus have a short product life cycle (Kök et al., 2009).

Products were classified according to the main protein ingredient being chickpeas, soybeans, other legumes than chickpeas/soybeans (e.g. lentils), sesame seeds or other seeds, or commercial/culinary name (= customary name as mentioned in the European law EC no.1169/2001) and mode of storage (Figure 2). The commercial named products couldn't be categorized based on the main ingredient due to the lacking link between these commercial names and a specific main ingredient. For example, 'pesto' and 'tapenade', culinary names, may have tomato as the main ingredient, but also leafy herbs or olives can be applied as the main ingredient. A classification based on the main ingredient would separate this commercial name into multiple subcategories. Contrarily, the product 'hummus' will always belong to the subcategory 'chickpeas', however not all chickpea products will be hummus.

Five subcategories with an equal abundance of products represented most of the VSD (72.5%): soybeans (17.3%), other seeds (14.9%), pesto (14.6%), tapenade (13.0%), and chickpeas (12.7%). In all subcategories (but salsa), products could be stored at either ambient temperature or refrigerated temperature with overall, the majority of the products were stored in ambient conditions (55.8%) (Figure 2). The subcategories chickpeas, soybeans, tapenade, and guacamole were mainly (>75.0%) stored in refrigerated conditions, the other subcategories were mainly (>66.7%) stored in ambient conditions. This is exemplifying the broad range of product formulation and preservation methods applied by the food manufacturers. In case a market study was performed in another time period of the year, potentially a slightly different data set could be retrieved due to the seasonality of the product offer in Belgium.

Of the many functional additive groups identified in the European law (EC no. 1333/2008), ten were present in the retrieved vegetarian spreads and dips dataset with multiple E-numbers being used for each functional additive group (Table 2). This indicates that many food producers are relying on the use of these preserving agents to control product safety and quality during the storage period. Acids (e.g. lactic acid, E270) and acidity regulators (e.g. sodium bicarbonate, E500) together with preservatives (e.g. potassium sorbate, E202.) were present in most of the subcategories. Lactic acid (E270) and citric acid (E330) were the most common food acids/acid regulators with an overall presence of respectively 33.8% and 41.8%. Potassium sorbate (E202) was by far the most applied food preservative (33.3%), although other preservatives dominated certain subcategories like E1105 (lysozyme) in pesto. Potassium sorbate causes an inhibitory effect on microorganisms in its non-dissociated form and will be most useful in products with low pH (Lambert & Stratford, 1999; Plumridge et al., 2004). Thickeners (e.g. xanthan gum, E415) were in 81.8% of the guacamoles present but were not applied in subcategories like pesto and sesame seeds-based products. Some subcategories contained more than eight different types of functional additive groups (e.g. products based on soybeans) while other subcategories contained less than four functional additive groups (e.g. products based on sesame seeds) (Table 2). This could be related to the intrinsic properties of the main ingredients which may require more/or fewer additive classes to obtain a reasonable (microbiological) product stability. The number of additives (E-numbers) found in each functional additive group could vary depending on the subcategory, ranging from eight (e.g. preservatives for product group based on chickpeas) to one (e.g. emulsifiers in guacamole) (Table 2). The European Commission has a database (AUTHORISATION OF ADDITIVES (europa.eu)) in which all the approved E-numbers are listed with the corresponding additive name and concentrations allowed to be used in different food categories.

Despite the abundant use of preservatives in many products, 35.8% of the products were classified as 'clean label'. Products based on sesame seeds, other seeds, and legumes (ex. chickpeas/soybeans) contained relatively the most 'clean label' products with an abundance of respectively 82.4%, 72.7%, and 60.0%. 'Guacamole' was the only subcategory that didn't contain any 'clean label' products as such with at least an antioxidant being present, most probably to suppress the enzymatic discoloration of avocado (Daiuto et al., 2011). A strict definition of 'clean label products' was used in this paper: only when the products contained no additives, they were labelled as 'clean label'. No global, commonly accepted definition is established of what 'clean label' means despite being on the market since the eighties. Products with claims such as 'free from additives/preservatives', 'no GMOs', 'natural', 'organic' can be perceived by consumers as 'clean label' (Asioli et al., 2017). Products with a simple ingredient list may also fall under the scope of 'clean label' (Aschemann-Witzel et al., 2019; Asioli et al., 2017; Ingredion, 2014). Food businesses are avoiding the use of difficult to pronounce, complex-sounding food additives, as these are perceived as more harmful by the consumers, and to use natural-sounding, familiar compounds (Aschemann-Witzel et al., 2019; Bahník & Vranka, 2017; Ingredion, 2014; Song & Schwarz, 2009). Producers might replace additives with natural ingredients that fulfil a similar function e.g. replacing citric acid with lemon juice or acetic acid with wine vinegar (FASFC, 2019). This was noticed in the label study of various so-called 'clean label' products like pesto and salsa, where these ingredients do not occur in common recipes.

In the case of processed food, the label 'organic' i.e. bio/eco is legally defined and means a minimum content of 95% of organic agricultural ingredients and strict conditions for the remaining 5%. Regulation no. 2018/848 defines the use of the term bio/eco i.e. which products are allowed to use the organic logo, and specific processing principles for the production of organic food. Remark that the use of additives is permitted, albeit restricted. In this study, 45.3% of the products were classified as 'organic' which is higher than the percentage of 'clean label' (35.8%) products. The subcategories 'sesame seeds', 'other seeds', and 'soybeans' contained relatively the most 'organic' products with an abundance of respectively 76.5%, 98.2%, and 81.3%. Not all 'clean label' products are 'organic' and not all 'organic' products are 'clean label' according to our definition of 'clean label'. However, the 'organic' products can be perceived as 'clean label' by consumers when not applying the strict definition of 'clean label' as used in this research.

3.2 Characterization of selected dips and spreads

3.2.1 Physicochemical characteristics of spread and dips in relation to microbiological growth potential

For both refrigerated samples as ambient samples, no clear increase or decline in pH was noticed between the different sampling times, day or purchase (DP) and end of shelf-life (ES), or after forcing tests of ambient stable samples. For the refrigerated samples, the pH ranges from 3.6-6.1 for DP and 3.7-6.1 for ES (Table 3). The standard deviation of the pH was low and a fairly uniform pH is present, besides products based on legumes (ex. chickpeas/soybeans) and soybeans where a higher pH (± 5.0 on average) and a wider range (SD > 0.60) was measured. The pH difference was larger between the different subcategories. Products based on legumes showed the highest pH (pH = 6.1 at ES) and also the highest standard deviation (0.8) and other spreads and dips with a vegetable or herb base showed the lowest pH (pH = 3.6 at DP). The following results can be obtained to the potential outgrowth of relevant pathogens (considering solely the pH as intrinsic factor) : growth of *Listeria monocytogenes* (minimum pH for growth 4.4 (Uyttendaele et al., 2018)), if present, was possible in 57.7% of the samples at the DP and in 60.0% of the samples at the ES. Growth of *Bacillus cereus* (minimum pH for growth 4.9 (Uyttendaele et al., 2018), if present and based solely on the pH, was possible in 19.3% of the samples at the DP and in 20.0% of the samples at the ES of which soybeans-based products (67.0%) were the most abundant. Only pH values for tofu and hummus were found in the Literature (Table 4). These pH ranges match with the ones found in our study both for hummus which is part of the subcategory 'chickpeas' and for tofu which is part of the subcategory 'soybeans'.

The average pH of ambient samples based on chickpeas (pH 4.2 ± 0.1), other seeds (pH 4.3 ± 0.1), legumes (ex. chickpeas/soybeans) (pH 4.2 ± 0.0), pesto (pH 4.3 ± 0.1), tapenade (pH 3.8 ± 0.1), salsa (pH 4.0 ± 0.1), guacamole (pH 3.8 ± 0.1) and other spreads and dips with a vegetable or herb base (pH 3.7 ± 0.5) remained below the minimum pH of growth for *Clostridium botulinum* (pH = 4.6 (Uyttendaele et al., 2018)). Only products based on sesame seeds (pH 6.0 ± 0.2) and soybeans (pH 4.8 ± 0.1) had pH values that exceeded this minimum growth pH.

The minimal water activity necessary for *Listeria monocytogenes* to grow is 0.92 (Uyttendaele et al., 2018). Except for one product from the subcategory 'other spreads and dips with a vegetable or herb base', all the other refrigerated samples exceed this water activity. Only two more samples have a water activity that is lower than 0.94, the minimum water activity for the growth of *Salmonella* spp., and pathogenic *E. coli*. This means that 96.2% of the investigated VSD contain a water activity that might allow the growth of different pathogenic microorganisms ($a_w > 0.95$). The ambient samples had, with exception of the subcategory sesame seeds, a water activity above 0.94 which is the limit for *Clostridium botulinum* (Uyttendaele et al., 2018).

Growth of pathogens like *Listeria monocytogenes* and *Bacillus cereus,* when present, was possible in many of the refrigerated samples based on pH and water activity. Taking into account the hurdle principle, fewer products could be providing growth habitats for organisms than solely based on pH and a_w. Temperature is also an important factor, especially for the refrigerated vegetarian spreads and dips which are kept at temperatures at which the growth potential is low or even not possible for some pathogens. Similarly, the applied gas composition also has an effect on the survival of pathogens (Debevere et al., 2021). For the ambient stored products, based on pH and water activity, the growth of pathogens like *Clostridium botulinum*, if present, was only possible in the two samples of the subcategory soybeans (with the pH as determining factor). This means that most of these products have besides the applied heat treatment at least one extra hurdle that prevents the growth of present pathogens.

The gas composition in the headspace was only measurable in 78.2% of the refrigerated samples and could not be analyzed for the ambient samples due to the used method. A needle is punctured through the packaging to measure the gas composition and the metal lid of the package of ambient stored products was not penetrable with the needle. The gas composition in the headspace was very variable. The average, median, minimum, maximum, and standard deviation of the CO_2- and O_2-concentration for each subcategory are given in Table 3. In 75.4% of measurable refrigerated samples, the difference in O_2-concentration between the DP and the ES was less than 5%. In 63.9% of the measurable samples, a decrease in O_2-concentration and in 57.4% an increase in CO_2-concentration was noticed. In 13.1% of the measurable refrigerated samples, the difference in CO_2-concentration between DP and ES was higher than 5% and was always an increase. Overall the subcategory 'guacamole" knew high concentrations of CO_2 (>20%, excl. one product with an CO_2- concentration of 6.5%) and low (< 2%) concentrations of O_2 on the day of purchase. Overall two extrema were noticed in the gas composition. First, the VSD could be packed with low amounts of oxygen (<2%) and high amounts of carbon dioxide (>10%) by the application of a modified atmosphere packaging as a preservation technique (Debevere et al., 2021). Carbon dioxide has an antimicrobial effect in a concentration of >10%. Aiding the effect is the increased solubility in refrigerated temperatures (Garcia-Gonzalez et al., 2007). Only subcategories pesto and guacamole had an average CO_2 concentration above 10%. Most of the other subcategories (besides the subcategories legumes (excl. chickpeas/soybeans), sesame seeds, and other seeds) contained products with CO_2 concentrations above 10%, indicating that this is a widely used preservation technique. Although scarce, studies have shown that elevated CO_2 concentrations aid in the preservation of VSD (Cosmai et al., 2017; Fabiano et al., 2000; Russo et al., 2014). In the contrast, the VSD could be packed under normal atmospheric conditions indicating that it is not necessary to apply a modified atmosphere packaging. Packaging under normal atmospheric conditions was not always clear because the purchased VSD were already several days old so gas exchange might have occurred during that time, giving a deviation from the standard atmospheric conditions (21% O_2, 78% N_2…).

3.2.2 Remaining shelf-life of vegetarian spreads and dips

Samples were purchased in supermarkets, as a representation of consumers' practices, and therefore the overall shelf-life given by the manufacturer is not traceable. The remaining shelf-life, i.e. the number of days between the moment of purchase and the expiration date indicated on the label was used to have an indication of the shelf-life for the consumer (Daelman et al., 2013). High variability was seen within and between the subcategories in the remaining shelf-life. Some subcategories had long remaining shelf-life periods (e.g. products based on soybeans), other subcategories had rather short remaining shelf lives (e.g. guacamole) (Figure 3). The average, mode, and standard deviation of the remaining shelf-life of the 78 examined refrigerated samples are respectively 32, 22, and 45 days and indicate a large variability in remaining shelf-life among the VSD on the market. Only 'guacamole' and 'other spreads and dips with a vegetable or herb base', harbor products that have a remaining shelf-life shorter than 5 days. The subcategory based on soybeans shows the highest average and standard deviation with respectively 87 and 54 days and 'guacamole' shows the lowest average, mode, and standard deviation with respectively 10, 7, and, 7 days. One sample of a refrigerated product based on soybeans stood out with a remaining shelf-life of 387 days and had a longer remaining date compared to some ambient products. The remaining shelf-life of the ambient samples always exceeded 4 weeks. As the samples were purchased in retail shops, no statements on the overall shelf-life given by the food producer can be made. According to the European law EU no. 1169/2011, the shelf-life should be a 'use by' date instead of a 'best before' date if the product is highly perishable and may cause harm to human health. No other legal protocols are provided to determine the kind of date (use by or best before) and shelf-life itself and it is thus the responsibility of the producer. The shelf-life itself can be based on various parameters such as pathogens, spoilage organisms, physicochemical changes, and sensorial changes (EFSA, 2020; Valero et al., 2012).

3.2.3 Microbiological composition of vegetarian spreads and dips

The refrigerated samples were purchased in duplo and kept at 7 °C with the first one being analyzed within a maximum of 2 days after purchase (=DP) and the second one approximately three days around the ES with one exception being analyzed six days after the ES (Figure 1). All samples of a particular food product were purchased in the same retail shops and had the same shelf-life date indicated on the package. Therefore, it is assumed that they are produced in the same production batch to avoid between-batch variability in the microbiological results. The high variability in cell count for quality indicators can be assigned to the different processing methods and/or additives and/or hygiene levels occurring during production (Table 5). High numbers of the investigated quality indicators may be due to high contamination of the raw ingredients, no or failed inactivation steps during processing, or due to unhygienic processing and cross-contamination (Uyttendaele et al., 2018). When averages of psychrotrophic total plate count and psychrotrophic lactic acid bacteria are compared (Table 5), it is clear that a big part of the psychrotrophic total plate count exists out of lactic acid bacteria. Products in subcategories ‘chickpeas’, ‘pesto’, ‘tapenade’, and ‘guacamole’ contain the highest average psychrotrophic total and lactic acid bacteria counts. Guacamole and tapenade have the highest average yeast and fungi counts on both DP and ES (Table 5). Guacamole is highly perishable as indicated by the short shelf-life and suitable pH, a_w for microbiological growth. This is confirmed in the rapid growth of microorganisms during storage. Noticeable is that at the end of shelf-life, sensorial deviation is possible for subcategories based on chickpeas, soybeans, pesto, tapenade, guacamole, and other VSD with a vegetable or herb base due to exceeding 5 x 10^7 CFU/g lactic acid bacteria or 3 x 10^5 CFU/g of yeasts which are the limits for sensorial detection (Uyttendaele et al., 2018). Sulfite reducing *Clostridia* never exceeded numbers higher than 3.6 log CFU/g.

Despite the growing interest in these types of products, so far, the microbial quality and safety of only hummus (Khiyami et al., 2011; Omar et al., 2005; Shehata & Alfaris, 2005; Yamani & Al-Dababseh, 1994) and tofu (Ashraf et al., 1999; Tuitemwong & Fung, 1991) were limited investigated (Table 4). The comparison between these studies (Table 4) and our findings (Table 5) is not fully correct. In the latter studies, the mesophilic plate count was determined instead of the psychrotrophic plate count applied in our study for the refrigerated samples as this is in correspondence with the storage temperature of prepacked dips and tapenades being 4-7 °C (Uyttendaele et al., 2018). Further, the mode of their production process is different: products resulting from restaurant settings are meant to be consumed fast, know a shorter shelf-life and no modified atmosphere packaging is applied. This is in contrast with the retail samples in our study (e.g. chickpea products have a remaining shelf-life of 3 weeks, Figure 3).

Omar et al. (2005) sampled products in two seasons, summer and winter, and noticed higher cell counts in the summer. In this study, the microbiological data were obtained in the fall-winter. However, seasonal variability is not expected for retail samples as the industrial production processes are standardized and variability in the microbiology is undesired. It is more the impact of different recipes and foods on the market which are linked to the seasons.

Tuitemwong et al. (1991) and Ashraf et al. (1999) both examined tofu from retail stores (Table 4), which is part of the subcategory soybeans in this study. The average and maximum cell counts obtained from these studies are higher compared to our results for soybean products (Table 5). Both studies used a different methodology compared to our study, with the determination of the mesophilic total plate count instead of the psychrotrophic plate count. Further, some analyzed products were already past their expiration date (Ashraf et al. (1999)). In our study, tofu was applied as an ingredient in the spreads and dips, which might explain the lower obtained cell counts.

A single agar was used in this research to measure the hygiene indicators (Table 1). Three samples contained coliforms (blue spots on RAPID *E. coli*), *E. coli* was not detected. Two of these samples were pesto with 2.9 and 3.6 log CFU/g at the DP and 1.8 and 2.7 log CFU/g at the ES. The third sample was a tapenade with 2.6 and 2.1 log CFU/g at the DP and the ES respectively. Only for hummus (part of the subcategory chickpeas), coliform and *E. coli* counts could be found in the Literature (Table 4). *E. coli* was not detected in 10 g in the study of Yamani & et al. (1994) and was detected, not quantified, in the studies of Khiyami et al. (2011), Shehata et al. (2005) and Omar et al. (2005). Coliforms were detected in the studies of Yamani et al. (1994) and Khiyami et al. (2011) (Table 4). The presence of the hygiene indicator coliforms and/or *E. coli* enhances the theory that products from restaurant settings are processed in a less hygienic way compared to retail samples.

In the refrigerated samples, *Listeria monocytogenes* was not detected in 10 g. This was also confirmed for hummus in the study of Omar et al. (2005). The highest cell count for *Bacillus cereus* was found in guacamole with 3.5 and 3.3 log CFU/g on the DP and the ES respectively, followed by tapenade with maximum counts of 3.0 and 2.8 log CFU/g on the DP and the ES respectively. For all the products, the average *Bacillus cereus* counts varied between 1.0 and 1.7 log CFU/g. *Bacillus cereus* thus never exceeded 5.0 log CFU/g, which is the limit at which disease symptoms may start to appear (Uyttendaele et al., 2018). The presence of *Bacillus cereus* in hummus was also confirmed but not quantified by Shehata et al. (2005).

The ambient stored samples contained low cell counts even after all forcing tests, i.e. after 7 days at 55 or 37 °C. Except for products based on chickpeas, sesame seeds, and other seeds, the ambient samples had cell counts lower than the limit of detection or lower than the limit of quantification for all investigated microbiological parameters, at 37 °C and 55 °C. The subcategory 'other seeds' had countable results >3 log CFU/g on the DP for one product for mesophilic total plate count (4.3 log CFU/g) and aerobic spores (5.1 log CFU/g). Chickpeas and sesame seeds-based products showed cell counts after the 7th day of the incubation period at 37 °C and 55 °C for total plate count and anaerobic spores but these values remained below 3 log CFU/g. These findings for sesame seeds were lower or within the range (2.0–5.9 log CFU/g) of the results obtained in the research of Khachfe et al. (2018). In our study, for the subcategories, 'sesame seeds' and 'other seeds', the mesophilic total plate count and aerobic spore count were of the same order of magnitude indicating that the mesophilic total plate count consisted partially of spores which can be due to the low water activity of the main ingredients (Khachfe et al., 2018; Salazar et al., 2019).

4. Conclusion

The consumers' interest in healthy food without additives combined with the increasing need for sustainable food systems make plant-based products, under which ready-to-eat vegetarian spreads and dips, gaining in popularity. Knowledge concerning product formulation and preservation methodology to guarantee microbiological stability during the shelf-life is lacking. The presented market research showed that these pre-packaged ready-to-eat foods are a broad food category with a range of plant-based main ingredients. Hurdle principle as preservation method resulted in a range of combinations of storage temperature (ambient or refrigerated), the use of additives, and physicochemical boundaries (pH, a_w, gas composition) and resulted in remaining shelf-lives ranging from under 5 days up to more than 4 weeks. Overall, ready-to-eat vegetarian spreads and dips were ambient stored, with large differences in product formulation between the subcategories : use of food acids/acid regulators and preservatives, a low pH or modified atmosphere packaging. The increasing interest in 'clean label' and organic products of European consumers is reflected with a large part of the retrieved products being labeled 'organic' and/or containing no additives at all. Although many food additives were found the presented market study, food producers reformulate their products towards ‘clean label’ without compromising food quality/safety to meet consumers’ needs in this fairly new product segment. The storage tests of the refrigerated samples revealed microbial growth of quality indicators which varied among the samples/subcategories in their remaining shelf-life and highlights the use of different preservation techniques. Ambient stored products showed, as expected, low microbiological counts due to the applied heat treatment in the production process. None of the products contained pathogenic microorganisms, in numbers that may harm food safety. However, pH and a_w were occasionally suitable for pathogenic growth if no other preservation technique would be used. The study demonstrates the wide range of available food products, formulations, and preservation modes in this novel and emerging vegetarian dips and tapenades.

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Figures

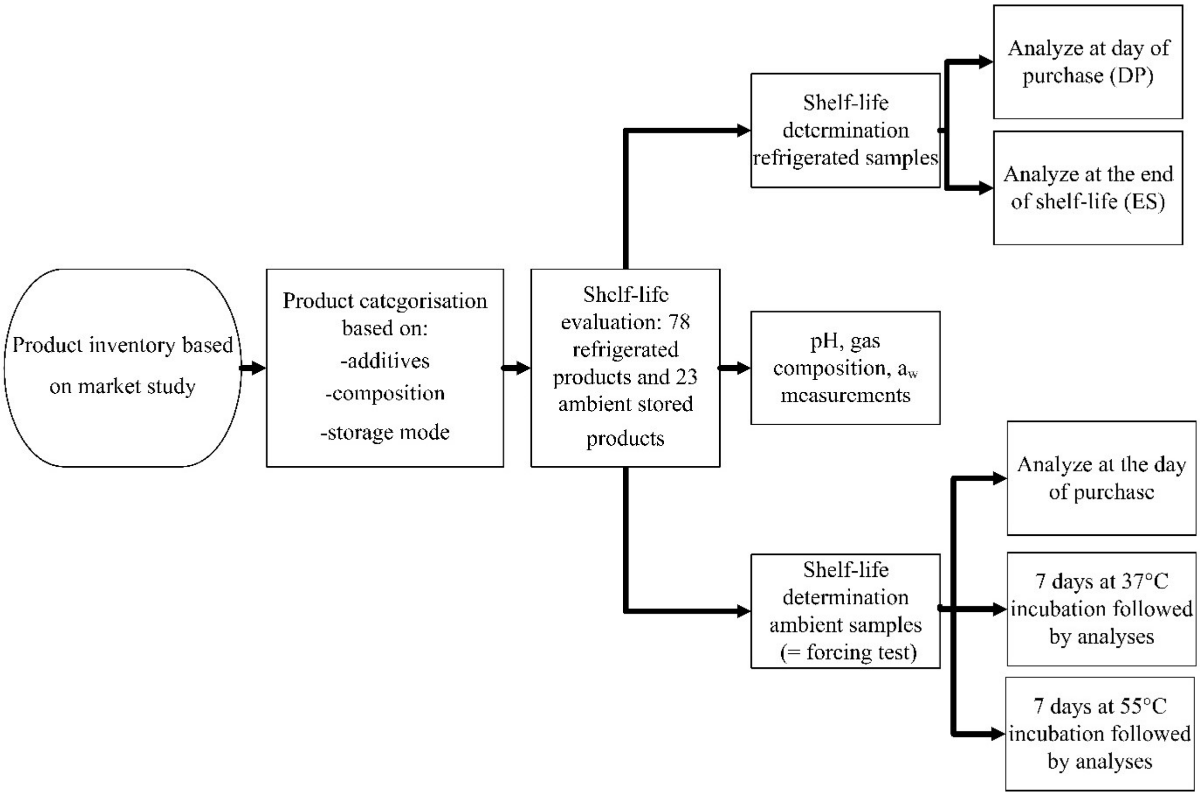

Figure 1: Applied research scheme for the product survey and characterization of vegetarian dips and spreads

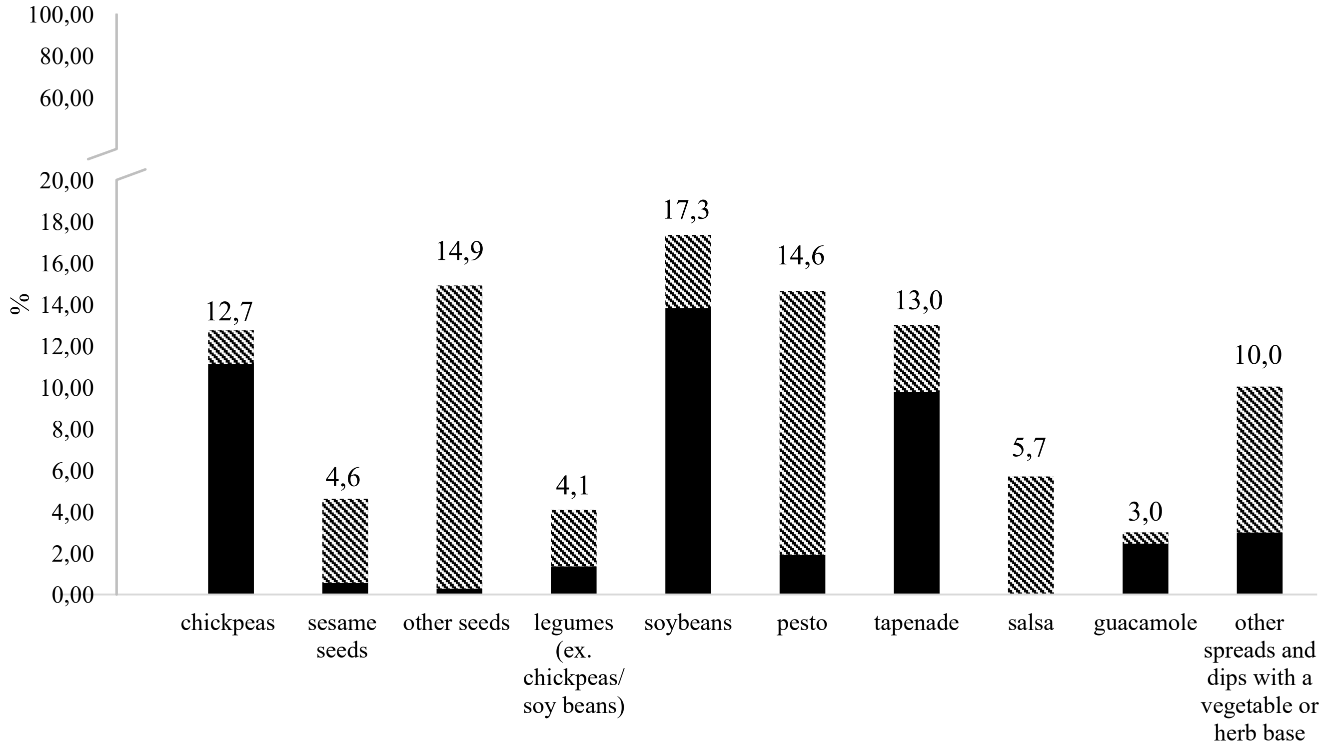

Figure 2: Categorization of 369 identified vegetarian spreads and dips from retail markets in Belgium according to main ingredient/commercial name. The relative numbers are given for the whole subcategory (above each column) and the different patterns indicate the % of products stored at ambient temperature (striped), and products stored at refrigerated temperature (black) within that subcategory.

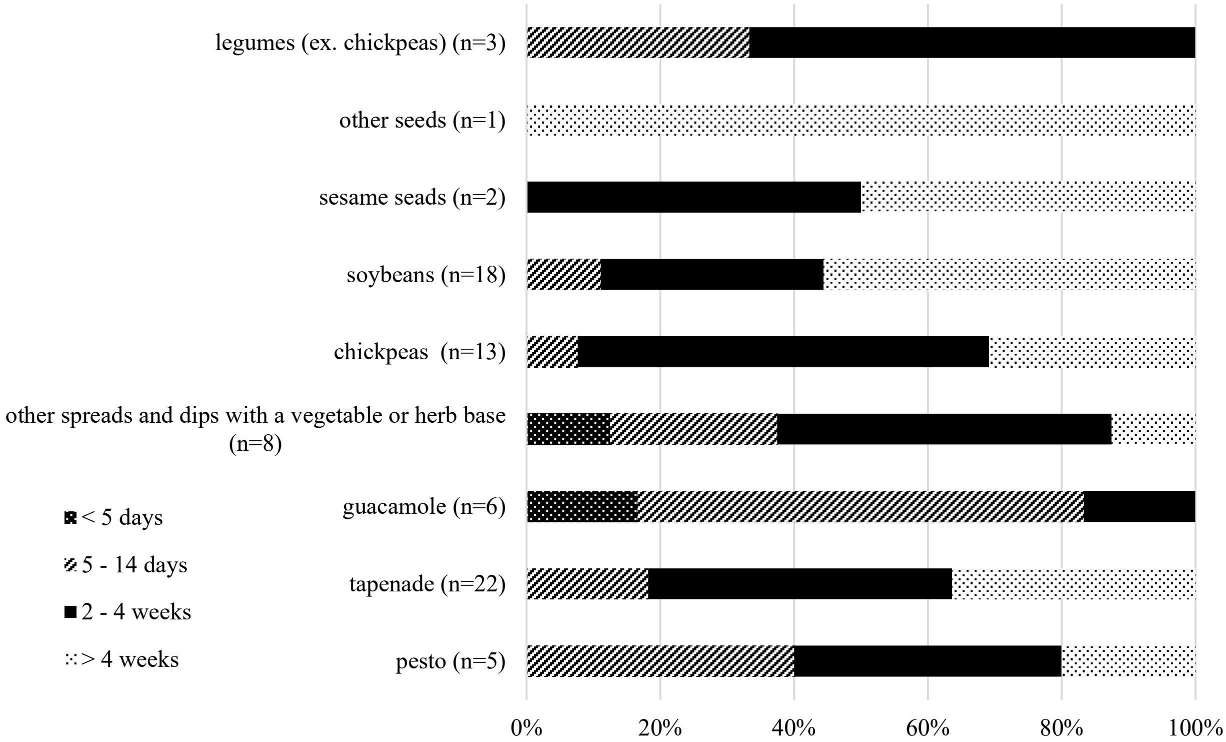

Figure 3: Relative distribution of remaining shelf-life (i.e. less than 5 days, between 5 and 14 days, between 2 and 4 weeks, more than 4 weeks) over the different subcategories for the 78 refrigerated stored vegetarian dips and spreads. For each subcategory the number of included products is given between brackets. The subcategory of “salsa” did not contain any refrigerated products so this no remaining shelf-life for refrigerated products can be given.

Tables

Table 1: Microbiological parameters analyzed as quality, hygiene indicator and pathogens for refrigerated and ambient stored dips and tapenades and applied analytical method (FAVV, n.d.; Pothakos et al., 2012; Uyttendaele et al., 2018).

	Indicator	Method	Media (agar)	Supplements	Incubation Time	Incubation Temperature	Refrige rated sample	Ambient sample
quality indicators	**Psychrotrophic total plate count**	Modified from ISO 6222:1999	PCA (CM0325, Oxoid)	None	4-5 days	22°C	x	
	Mesophilic total plate count	ISO 4833-1:2013			3 days	30°C		x
	Psychrotrophic lactic acid bacteria	ISO 15214:1998	MRS, (CM0361, Oxoid)	None	4-5 days	22°C	x	
	Mesophilic lactic acid bacteria				3 days	30°C		x
	Psychrotrophic yeast and fungi count	AFNOR NF V 08-059	YGC, (3564104, Biorad)	None	4-5 days	22°C	x	
	Mesophilic yeast and fungi count				3 days	30°C		x
	Sulfite reducing *Clostridia*	ISO 15213:2003	TSC, (CM0587, Oxoid)	Perfringens selective supplement (SR0088, Oxoid)	24-48h	37°C	x	x
	Anaerobic spores	Heat treatment 10 min 80°C ISO 15213:2003						x
	aerobic spores	Heat treatment 10 min 80°C ISO 4833-1:2013	PCA, (CM0325, oxoid)	None	3 days	30°C		x
hygiene indicators	***E. coli* and coliforms**	AFNOR BRD-07/8-12/04	REC, (3564024, Biorad)	None	24h	37°C	x	
pathogens	***Bacillus cereus***	ISO 7932:2004	MYP, (CM0929, Oxoid)	Polymyxin B (SR0099, Oxoid) Egg yolk Emulsion (SR0047, Oxoid)	24-48h	30°C	x	x
	***Bacillus cereus* spores**	Heat treatment 10 min 80°C ISO 7932:2004			24-48h	30°C		x
	Listeria monocytogenes	ISO 11260-1:2017 and ISO 11260-2:2017	ALOA, (3564043 Biorad)	AL supplement 1 (3564041, Biorad) (AL supplement 2, 3564042, Biorad)	24-48h	37°C	x	

Table 2: Additives found in the different subcategories of vegetarian spreads and dips (n = 369) scaled to the total amount of products containing at least one additive within the subcategory. The E-numbers identified on the list of ingredients, clean label and organic labelled products are indicated. The name of the additives corresponding to the E-numbers can be found in the database of the European Commission (AUTHORISATION OF ADDITIVES (europa.eu))

	pesto (n=54)	tapenade (n=48)	guacamole (n=11)	salsa (n=21)	other spreads and dips with a vegetable or herb base (n=37)	chickpeas (n=47)	soybeans (n=64)	legumes (ex. chickpeas/ soy beans) (n=15)	sesame seeds (n=17)	other seeds (n=55)
% Clean label (scaled to subcategory)*	**16.7**	**12.5**	**0.0**	**57.1**	**43.2**	**10.6**	**32.8**	**60.0**	**82.4**	**72.7**
% Organic label (scaled to subcategory)**	**14.8**	**0.0**	**9.1**	**4.8**	**35.1**	**29.8**	**81.3**	**73.3**	**76.5**	**98.2**
Acids and acidity regulators	**72.2** E270, E330, E500, E575	**70.8** E260, E270, E300, E327, E330	**27.3** E270, E330	**14.3** E270	**43.2** E260, E270, E300, E330, E501	**78.7** E260, E270, E330	**20.3** E260, E270, E330	**13.3** E260, E270, E330	**17.7** E270, E330	**5.5** E330
Preservatives	**25.9** E200, E202, E211, E224, E251, E1105	**62.5** E200, E202, E210, E211, E223, E224, E251	**27.3** E202, E211	**4.8** E202	**21.6** E202, E211, E228	**70.2** E200, E202, E210, E211, E223, E224, E330, E1105	**9.4** E202, E211, E262, E270, E325,	**2** E202, E223	**5.9** E202	\
Colors	**5.6** E150d, E160a	**31.3** E120, E160a, E160b, E160c, E579	**18.2** E133, E160a, E414	**4.8** E160c	**13.5** E160a, E160b, E160c	**12.8** E150d, E160c	**6.3** E100, E101, E140, E160a, E160c, E163	\	\	\
Antioxidants	**11.1** E300, E301, E304, E330	**12.5** E300, E330, E392	**100.0** E300, E330	**23.8** E300, E330	**13.5** E300	**23.4** E300, E304, E306	**3.1** E300, E304	\	\	**1.8** E300
Emulsifiers	**13.0** E322, E471, E472a	**2.1** E339	**9.1** E472e	\	\	**4.3** E471	**1.6** E322	\	\	\
Thickening agents	\	**39.6** E412, E415, E466	**81.8** E401, E414, E415	**4.8** E415	**24.3** E410, E412, E415	**36.2** E412, E415, E466	**31.3** E410, E412, E415	**20.0** E412, E415	\	**25.5** E410, E412, E415
Stabilising agents	\	**10.4** E509, E579	**27.3** E401, E412, E415	\	**2.7** E509	**8.5** E412, E415	**9.4** E407, E412, E415	**6.7** E412, E415	\	\
Emulsifiying salts	\	**10.4** E339	**9.1** E339	\	**2.7** E339	\	\	\	\	\
Gelling agents	\	\	\	\	\	\	**1.6** E440	\	\	\
Firming agents	\	\	\	\	\	\	**48.4** E509, E511, E516	**6.7** E509	\	\

* the percentage clean label products (scaled to the total number of products within the subcategory).

** the percentage organic products (scaled to the total number of products within the subcategory).

Table 3: Average, median, standard deviation, minimum and maximum of pH and gas composition in the headspace (% O_2 and % CO_2) of the sampled refrigerated vegetarian spreads and dips at the day of purchase (DP) and end of shelf-life (ES).

		pesto (n = 5)		**tapenade (n = 22)**		**guacamole (n = 6)**		**other spreads and dips with a vegetable or herb base (n = 8)**		**chickpeas (n = 13)**		**soybeans (n = 18)**		**legumes (ex. chickpeas/ soy beans) (n = 3)**		**sesame seeds (n = 2)**		**other seeds (n = 1)**	
		DP	ES	DP	ES	DP	ES	DP	ES	DP	ES	DP	ES	DP	ES	DP	ES	DP	ES
pH	average	4.73	4.76	4.17	4.18	4.44	4.48	4.18	4.25	4.75	4.76	5.11	5.15	4.95	4.95	4.24	4.21	4.97	4.92
	median	4.79	4.81	4.04	4.11	4.41	4.31	4.21	4.32	4.73	4.73	5.14	5.16	4.67	4.62	4.24	4.21	4.97	4.92
	Standard deviation	0.32	0.30	0.33	0.29	0.23	0.44	0.34	0.31	0.32	0.33	0.60	0.61	0.77	0.78	0.06	0.03	/	/
	minimum	4.39	4.45	3.88	3.78	4.13	4.09	3.63	3.65	4.32	4.28	4.19	4.17	4.38	4.45	4.20	4.19	4.97	4.92
	maximum	5.04	5.13	5.22	4.82	4.72	5.10	4.70	4.58	5.70	5.70	5.89	6.05	6.09	6.11	4.28	4.23	4.97	4.92
O_2-concentration	average	6.33	2.75	8.90	6.70	0.76	3.97	8.05	9.37	13.51	10.38	10.45	11.79	16.45	15.84	16.10	13.85	3.01	20.00
	median	2.22	0.35	10.90	4.90	0.67	0.69	4.09	10.80	19.50	11.10	6.96	8.91	19.00	19.30	16.10	13.85	3.01	20.00
	Standard deviation	8.96	4.26	6.98	5.86	0.82	4.95	8.36	6.97	8.14	7.70	6.78	7.17	5.67	6.44	0.57	2.47	/	/
	minimum	0.16	0.25	0.10	0.00	0.00	0.01	0.09	0.00	1.80	0.07	2.44	0.83	9.95	8.41	15.70	12.10	3.01	20.00
	maximum	16.60	7.67	18.50	16.70	1.61	9.65	19.20	17.30	20.40	19.80	20.40	20.60	20.40	19.80	16.50	15.60	3.01	20.00
CO_2-concentration	average	23.80	22.93	6.89	7.56	27.08	46.16	8.73	19.03	6.33	8.25	4.59	4.84	0.87	0.87	1.55	2.20	6.20	1.40
	median	28.90	28.50	5.60	6.05	23.70	37.50	8.50	10.80	1.30	4.90	4.40	3.00	1.10	0.90	1.55	2.20	6.20	1.40
	standard deviation	19.56	14.85	5.39	6.42	16.57	28.17	7.41	31.02	9.57	9.26	4.27	6.45	0.49	0.25	0.64	2.26	/	/
	minimum	2.20	6.10	1.00	1.10	6.50	21.50	1.10	1.40	0.30	0.30	0.30	0.30	0.30	0.60	1.10	0.60	6.20	1.40
	maximum	40.30	34.20	18.35	22.50	56.90	92.10	17.90	88.40	29.00	29.90	15.50	22.10	1.20	1.10	2.00	3.80	6.20	1.40

Table 4: Studies investigating hummus and tofu. Only the (average) pH was determined in these studies as physicochemical parameter. Several microbiological parameters are analyzed in these studies of which only the average lactic acid bacteria, the average total plate count coliforms and the average yeast and fungi count are given in log CFU/g.

	Study		pH	Lactic acid bacteria (log CFU/g)	Total plate count (log CFU/g)	Yeast and fungi (log CFU/g)	Coliforms (log CFU/g)
Hummus	(Yamani & Al-Dababseh, 1994)	*Restaurant: Winter*	4.5 – 6.8	3.0 – 8.5	3.9 – 8.5	0.4 – 5.0	0.6 – 4.4
		Restaurant: Summer	4.3 – 7.0	4.7 – 9.0	4.7 – 9.0	2.5 – 5.8	2.6 – 6.8
		Freshly prepared	4.2 – 4.6	1.1 – 2.2	2.1 – 2.8	<1-1.3	<1.0
	(Khiyami et al., 2011)	*Restaurant*	/	/	5.3	/	4.0 – 5.0
		Homemade	/	/	4.3 - 4.9	/	3.3 - 3.7
	(Omar et al., 2005)	*Restaurant*	4.4 – 6.8	/	5.2-6.7	2.1 – 3.3	2.3 – 3.1 [I]
Tofu	(Tuitemwong & Fung, 1991)	*Grocery store: tofu: Day 1*	4.5 – 6.2	/	3.4 - 6.2	/	/
		Grocery store: tofu: Day 30	5.2 – 6.3	/	8.0 – 9.7	/	/
		Grocery store: tofu juice: Day 1	4.2 - 6.5	/	2.2 - 6.7	/	/
		Grocery store: tofu juice: Day 30	4.2 – 6.4	/	7.0 – 8.8	/	/
	(Ashraf et al., 1999)	*Grocery store* [II]	4.8 – 6.4	/	1.5 – 7.9	/	0.9 - 4.9

[I] Enterobacteriaceae were analyzed

[II] different remaining shelf-lives were incorporated here ranging from 39 days to 10 days past expiration date

Table 5: Average, standard deviation, median, minimum and maximum of microbiological counts of quality indicators of the 78 examined refrigerated vegetarian spreads and dips, expressed as log CFU/g (LOD = 1.00 log CFU/g), at the day of purchase (DP) and at the end of shelf-life (ES).

		pesto n = 5		**tapenade n = 21**		**guacamole n = 6**		**other spreads and dips with a vegetable or herb base n = 8**		**chickpeas n = 13**		**soybeans n = 18**		**legumes (ex. Chickpeas) n = 3**		**sesame seeds n = 2**		**other seeds n = 1**	
		DP	ES	DP	ES	DP	ES	DP	ES	DP	DP	ES	DP	ES	ES	DP	ES	DP	ES
Total psychrotrophic plate count	average	4.1	5.9	4.7	5.5	5.3	7.6	4.1	4.0	3.7	3.5	3.4	2.7	2.7	5.8	2.8	2.9	2.9	2.4
	median	4.3	5.2	5.1	5.2	4.9	7.8	3.9	4.0	3.7	3.2	2.8	2.7	2.4	6.1	2.8	2.9	2.9	2.4
	standard deviation	1.3	1.6	1.6	2.1	1.2	1.2	1.1	1.7	1.3	1.4	2.4	0.3	1.6	2.2	0.4	1.2	/	/
	minimum	2.2	4.8	2.0	1.0	3.9	6.1	2.9	2.0	1.0	1.0	1.0	2.5	1.0	1.0	2.5	2.0	2.9	2.4
	maximum	5.5	7.8	7.3	9.4	7.1	8.9	6.1	6.4	6.0	6.0	8.9	3.0	4.7	8.2	3.1	3.7	2.9	2.4
Psychrotrophic lactic acid bacteria	average	3.6	4.1	3.9	4.3	5.3	7.6	3.3	3.5	2.7	2.2	2.4	1.5	2.5	5.5	1.4	2.5	1.0	1.0
	median	4.2	3.7	4.3	4.3	5.4	8.1	2.6	2.6	2.5	1.0	1.0	1.2	2.1	6.0	1.4	2.5	1.0	1.0
	standard deviation	1.6	1.5	1.9	2.0	1.6	1.5	1.9	2.3	1.3	1.6	2.2	0.9	1.6	2.3	0.6	2.1	/	/
	minimum	1.0	2.9	1.0	1.0	2.9	5.0	1.6	1.8	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	maximum	5.2	6.4	7.2	8.2	7.6	8.8	6.5	7.4	5.0	5.9	7.0	2.9	4.8	8.1	1.8	3.9	1.0	1.0
Yeast and moulds	average	2.7	3.1	3.1	3.0	3.1	4.4	2.8	2.8	2.8	2.3	2.5	2.0	2.0	3.7	2.0	2.0	2.0	2.0
	median	2.5	2.0	2.0	2.0	2.5	6.2	2.0	2.0	2.3	2.0	2.0	2.0	2.0	3.6	2.0	2.0	2.0	2.0
	standard deviation	1.0	2.0	1.7	1.4	1.3	2.7	1.2	1.5	1.1	0.8	1.8	/	/	1.6	/	/	/	/
	minimum	2.0	2.0	2.0	2.0	2.0	1.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
	maximum	4.4	6.6	6.1	5.9	5.3	6.7	4.6	5.7	5.0	5.5	9.5	2.0	2.0	6.1	2.0	2.0	2.0	2.0
Sulfite reducing *Clostridia*	average	1.2	1.3	1.3	1.3	1.2	1.1	1.2	1.4	1.5	1.1	1.1	1.0	1.0	1.0	2.0	1.0	1.0	1.0
	median	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	2.0	1.0	1.0	1.0
	standard deviation	0.4	0.7	0.6	0.8	0.3	0.3	0.4	0.9	1.0	0.6	0.2	/	/	/	1.5	/	/	/
	minimum	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	maximum	1.9	2.6	3.3	3.7	1.9	1.7	2.2	3.5	3.3	3.3	1.8	1.0	1.0	1.0	3.1	1.0	1.0	1.0