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Comparative study of minimally processed and fresh cole, according to microbiological quality issues

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Abstract

Vegetables are part of the complementary feeding of human beings. Cole is very appreciated by Brazilians, and it is easy to grow and readily available throughout the year in street markets and supermarket shelves. Marketed both fresh and minimally processed, cole is an important means of contamination by pathogenic microorganisms. It causes foodborne diseases (FD) if not properly sanitized. Thus, this study aims to analyze the microbiological quality of minimally processed cole and fresh cole, comparing their respective levels of contamination. For this purpose, cole samples were collected in tents located in two corridors of a street food market in Vitória da Conquista-BA, Brazil. The petrifilm technique was used for analysis of aerobic mesophiles, *Staphylococcus aureus*, total coliforms, molds and yeasts. All the analyzed samples were contaminated with respect to the examined microorganisms; therefore, both fresh and minimally processed cole are unfit for human consumption unless proper cleaning and sanitizing techniques are not applied.

Key words: Brassica. Microbiological Analysis. Food Microbiology. Food Contamination.

Introduction

Cole (Brassica oleracea) is a vegetable native to the Mediterranean area, and belong to the family of Brassicas. There is a huge demand for it in mid-sized and large cities. It is easy to grow and readily available all year round. It is rich in vitamins, minerals and fiber; it has high levels of iron and vitamin C, which favors the absorption of this mineral in the body.[1-3]

There is great interest in the production of minimally processed fruits and vegetables, as a result of changes in people's lifestyle: e.g., reduced time available for preparing food, especially vegetables, both within the family household and in food and nutrition units. For consumers, food is supposed to be healthy, easy to prepare and safe to eat. In Brazil, there are several minimally processed food products. Cole is highly consumed by the Brazilian population; therefore, they are frequently used in minimally processed form.[4,5]

According to Gomes et al.,[6] minimally processed vegetables are products previously prepared by peeling, cutting, sanitization, centrifugation and packaging, in order to keep the product in its fresh state, while adding functionality and value attributes to it.

Although there are many benefits, there is wide debate on the safety of these products, given the incidence of spoilage and pathogenic microorganisms that transmit diseases, and product loss. As a result of poor handling and mechanical injuries, minimally processed products are susceptible to contamination, which in turn accelerates degradation and loss of quality.[7]

Safe food is consumers' guarantee to purchase a quality product of their choice, while adding health and safety attributes to it. However, substances that can harm human health cannot be seen externally at the time of purchase.[5]

The microbiological quality of minimally processed foods is associated with the presence of spoilage microorganisms, which adversely change the characteristics of food, such as undesirable sensory changes (color, flavor, aroma), as well as the concentration of pathogenic microorganisms to amounts that may cause damage to consumer health. Therefore, microbiological safety is the absence of microbial toxins and microorganisms that cause foodborne illnesses, such as foodborne infections, toxinfections and intoxications.[8] Minimally processed foods are a means conducive to microbial growth due to loss of integrity of the product, resulting in damaged tissues with high moisture content in vegetables.[5]

This study aimed to compare minimally processed and fresh cole, based on microbiological quality aspects, given the increased consumption of minimally processed vegetables, the microbiological quality of these products and the concern about the occurrence of foodborne diseases (FD), as well as the fact that cole is a frequently consumed vegetable by Brazilians.

Method

The study was conducted in a food supply center (CEASA) in downtown Vitoria da Conquista, Bahia, where ten grocery stalls were randomly selected for sample collection. Five stalls were in a corridor and five in another corridor, which were classified as corridor 1 and corridor 2 (C1 and C2). A fresh cole sample and a minimally processed cole sample were collected in each stall, totaling ten samples for each product.

Data collection was conducted in two replications, with a two-day interval for each collection, both under aseptic conditions. After collection, the samples were sent to the laboratory in thermal bags with ice pack, and kept refrigerated until the time of analysis. Microbiological testing of the cole samples was performed at the microbiology laboratory at the Faculdade de Tecnologia e Ciências (*School of Technology and Sciences*, FTC) of Vitória da Conquista, Bahia. All methods followed the guidelines of Instituto Adolfo Lutz[9] and Silva et al.[10]

Samples were collected in two corridors (C1, C2); five minimally processed cole samples and the same amount of fresh cole samples. Pools of samples were made for each corridor, removing 25g of each sample, with total weight of 125 g.

Each pool of sample was homogenized, and 50 g was removed and diluted in 450 ml 1% sterile buffered peptone water - dilution that corresponds to 10^{-1}. Because the first analyses of aerobic mesophiles and *Staphylococcus aureus* show countless values, the samples were rediluted at 10^{-2} - dilution made by collecting 1 ml sample at 10^{-1} and diluting it in 9 ml sterile peptone water in a 50 ml erlenmeyer flask.

The samples were inoculated in Petrifilm plates for aerobic mesophiles, *Staphylococcus aureus*, total coliforms, molds and yeasts. The procedure was performed by rising the film from the plate, where 1 ml of the dilution was added at the center, and then covering it. The diffuser was positioned at the center of the plate to distribute the samples evenly. They were incubated in an oven at ± 38 ° C for 48 hours.

Results and discussion

In the present study, the analyzed cole samples were shown to be contaminated with high microbial counts ranging between 10^2 and 10^5 CFU/g; therefore, it may fall short of standards for consumption. Normative Rule RDC nº12/2001 by ANVISA (Brazilian Health Surveillance Agency)[11] does not set microbiological parameters for the assessed microorganisms; parameters were determined only for *Salmonella* and fecal coliforms.

All study microorganisms were found in the samples: (aerobic mesophiles, *Staphylococcus aureus*, total coliforms, molds and yeasts) compared with the sectors (C1 and C2) where they are marketed (Tables 1 and 2).

Table 1. Microbiological values (CFU/g) of minimally processed cole in its respective sectors. Vitória da Conquista-BA, 2014.

Location/ Replicate	Aerobic Mesophiles (10^{-2})	*Staphylococcus aureus* (10^{-2})	Total Coliforms (10^{-1})	Molds and Yeats (10^{-1})
C1 R1	Countless	$1.2.\ 10^4$	Countless	$2.25.\ 10^2$
C1 R2	Countless	$7.2.\ 10^4$	$8.7.\ 10^3$	$1.9\ .10^2$
C2 R1	Countless	$8.25.\ 10^3$	$5.8.\ 10^3$	$5.25.\ 10^2$
C2 R2	Countless	$7.2.\ 10^4$	$1.68.\ 10^4$	$3.25.\ 10^2$

C1 and C2 (Collection sectors of the samples); **R1 and R2** (Replicates).

Table 2. Microbiological values (CFU/g) of fresh cole in its respective sectors. Vitória da Conquista-BA, 2014.

Location/ Replicate	Aerobic Mesophiles (10^{-2})	*Staphylococcus aureus* (10^{-2})	Total Coliforms (10^{-2})	Molds and Yeats (10^{-2})
C1 R1	1.96. 10^5	2 .10^3	1.4. 10^4	2 .10^2
C1 R2	1.3. 10^5	1.5. 10^4	4.3. 10^3	1.9. 10^2
C2 R1	1.65. 10^5	5.25 .10^3	9.8. 10^3	1.7. 10^2
C2 R2	1.6 .10^5	53 .10^2	9.35 .10^3	1.65 .10^2

C1 and C2 (Collection sectors of the samples); **R1 and R2** (Replicates).

The analysis of aerobic mesophiles in minimally processed cole samples presented countless results in both C1and C2. The Normative Resolution RDC nº 12/01-ANVISA[11] does not determine acceptable parameters for aerobic mesophiles, but according to Franco and Landgraf,[12] the high count of this group of bacteria indicates the use of contaminated raw material and poor processing. Ravelli et al.[13] detected microbial contamination in minimally processed vegetables, ranging from 1.0 .10^7 to 7.3 .10^3 CFU/g for aerobic mesophiles.

Sthaphylococcus aureus showed high microbial count in all samples, indicating potential health hazard due to the possible presence of staphylococcal enterotoxins.[12] Identical values of *S. aureus* were found in the second replicate for C1 and C2. However, the minimum value occurred in the first sample for C2. The mean value found in all samples corresponded to 4.1 .10^4 UFC/g. According to Forsythe,[14] toxins are produced in contaminated food with values ≥ 10^5 of *S. aureus*, leading to symptoms of staphylococcal infection.

Research conducted by Assis & Uchida[15] showed values > 1 .100 NMP/g total coliforms in sliced cole sold in supermarkets in Campo Mourão-PR, but in the present study, the values were higher in all samples, and were countless in the first sample in C1. The Normative Resolution RDC nº 12/01-ANVISA[11] establishes acceptable levels only for thermotolerant coliforms.

As for yeasts and molds, samples were more contaminated in C2, with a mean value of 3.1. 10^2 UFC/g. Higher values between 4.1 .10^4 and 3,7 .10^6 UFC/g were present in carrots and cabbage shredded sold in supermarkets in the city of Fortaleza-CE, as reported in research conducted by Bruno et al.[16]

Fresh cole samples (Table 2) tested positive for aerobic mesophiles, *Staphylococcus aureus*, total coliforms, molds and yeasts. However, the minimally processed cole samples were more contaminated from a microbiological point of view, compared with the fresh cole samples.

Table 2 shows the aerobic mesophile count, with a means of 1.6. 10^5 CFU/g fresh cole; the results were not obtained for minimally processed cole because of the large amount of bacteria found. According to Mogharbel,[17] foods whose surface is cut are most susceptible to attacks by microorganisms. They are present in the plant tissue itself or stem from the soil and air; additionally, excessive handling and release of nutrients in the cutting process favors possible contamination.

As regards the fresh cole samples contaminated by *Staphylococcus aureus*, the means found across sectors and replicates was 6.9 .10^3 CFU/g, and the pool of samples with greater number of colonies was in C1 in the second replicate. The presence of this microorganism indicates contamination by handling and poor sanitation conditions of the surfaces in contact with food.[12] However, the minimally processed cole sample was less contaminated; as shown in Table 1, there was a mean value of 4.1 .10^4 UFC/g of *Staphylococcus aureus*.

The total coliform values in the fresh cole samples were higher compared with those of minimally processed cole, with a mean of 9.3 .10^3 CFU/g, while the results for the minimally processed cole samples had a mean of 7.8 .10^3 UFC/g. According to Mogharbel,[17] the group of total coliforms is quite common in food, because they originate from the cultivation soil itself and are involved in the normal microbiota if grown in soil with feces. Thus, they are indicators of hygiene in the above-mentioned processes, and when they are found at high levels, they are indicative of risk of transmission of other pathogens.

Molds and yeasts were present in all samples shown in Table 2, in a mean amount of 1.8 .10^2 CFU/g. In comparison with Table 1, there is lower contamination level by these organisms. Minimally processed cole samples showed a mean of 3.1 .10^2 CFU/g. As stated by Mogharbel,[17] the presence of molds and yeasts in considerable numbers is indicative of moisture absorption in food processing.

Table 3 shows a comparison of mean microbiological values found in minimally processed and fresh cole samples, collected in C1 and C2, in two replicates.

Table 3. Comparative microbiological analysis (CFU/g) of fresh and minimally processed cole samples.Vitória da Conquista-BA, 2014.

Cole Samples	Location/ Replicate	Aerobic Mesophiles (10^{-2})	*Staphylococcus aureus* (10^{-2})	Total Coliforms (10^{-1})	Molds and Yeats (10^{-1})
Processed	C1,2/R1,2	Countless	$4.1.\ 10^{4}$	$7.8.\ 10^{3}$	$3.1.\ 10^{2}$
Fesh	C1,2/R1,2	$1.6.\ 10^{5}$	$6.9.\ 10^{3}$	$9.3.\ 10^{3}$	$1.8.\ 10^{2}$

C1,2 (location); **R1,2** (replicates).

The two cole samples (minimally processed and fresh) were contaminated with high microbial counts ranging from 10^{2} to 10^{5} CFU/g (Table 3). However, except for total coliforms, there was a slight increase in the values for the minimally processed cole samples. According to Bruno et al.,[16] contamination of minimally processed food products may occur during the slicing and cutting operations because of the presence of pathogens on the surfaces of the raw material or on the hands of handling staff. Thus, handling equipment in unsatisfactory hygiene conditions, combined with increased tissue damage, may favor microbial populations in vegetables.

Conclusion

It was concluded that the marketed cole samples are unfit for consumption because there was a large amount of microorganisms in them.

The microorganisms analyzed in this study can be considered as indicative of lack of hygienic-sanitary control, and there fundamental changes that should be made in respect to handling, storage and packaging of these products.

The population should be informed about the importance of washing and sanitizing vegetables properly before consumption, which contributes to the reduction or eradication of these microorganisms, and prevents the risks of foodborne diseases.

References

1. Empresa Brasileira de Pesquisa Agropecuária-Embrapa. Couve [Internet]. 2006. Acesso em: 05 abr. 2014. Disponível em: http://www.cnph.embrapa.br/hortalicasnaweb/couve.html
2. Moretti CL, Carnelossi MAG, Silva EO, Puschmann R. Processamento mínimo de couve. Brasília: Embrapa Hortaliças, 2000. 3 p. Comunicado Técnico, 13. Acesso em: 03 abr. 2014. Disponível em: http://bbeletronica.cnph.embrapa.br/2000/cot/cot_13.pdf
3. Oliveira-Calheiros K, Canniatti-Brazaca SG, Souza MC. Avaliação da disponibilidade do ferro em dieta complementada com couve manteiga. Alim. Nut. 2008; 19(1):37-42.
4. Carnelossi MAG, Silva EO, Campos RS, Soares NFF, Minim VPR, Puschmann R. Conservação de folhas de couve minimamente processadas. Revista Brasileira de Produtos Agroindustriais 2002; 4(2):149-155.
5. Santos APR, Junqueira AMR. Gestão da qualidade na couve minimamente processada no distrito federal: o caso da agroindústria Machadinho. Revista Brasileira de Produtos Agroindustriais 2012; 14(4):337-352.
6. Gomes CAO, Alvarenga ALB, Freire Junior M, Cenci SA. Hortaliças minimamente processadas. Brasília: Embrapa Informação Tecnológica; 2005. 34 p.
7. Moretti CL. Manual de processamento mínimo de frutas e hortaliças. Brasília: Embrapa Hortaliças; 2007.
8. Vanetti MCD. Segurança microbiológica em produtos minimamente processados. In: Resumos do 3º Encontro nacional sobre processamento mínimo de frutas e hortaliças 2004, Viçosa, MG. Viçosa: UFV; 2004. p. 30-32.
9. Instituto Adolfo Lutz. Normas analíticas do Instituto Adolfo Lutz. Métodos químicos e físicos para análise de alimentos. São Paulo: IMESP; 2008.
10. Silva N, Junqueira VCA, Silveira NFA, Taniwaki MH, Santos RFS, Gomes RAR. Manual de métodos de análise microbiológica de alimentos e água. 4ª ed. São Paulo: Livraria Varela; 2010.
11. Brasil. Resolução RDC n. 12, de 02 de janeiro de 2001. Aprova o regulamento técnico sobre os padrões microbiológicos para alimentos. Diário Oficial da União, 10 jan. 2001.
12. Franco BDGM, Landgraf M. Microbiologia dos alimentos. São Paulo: Atheneu; 2008.
13. Ravelli MN, Novaes APT, Nascimento GGF. Análise microbiológica de hortaliças minimamente processadas e comercializadas no município de Piracicaba, SP. Revista Higiene Alimentar 2010; 24(184/185):110-114. Acesso em: 01 abr. 2014. Disponível em: http://www.unimep.br/phpg/mostraacademica/anais/4mostra/pdfs/21.pdf
14. Forsythe SJ. Microbiologia da segurança alimentar. Porto Alegre: Artmed; 2002.

15. Assis LLR, Uchida NS. Análise da qualidade microbiológica de hortaliças minimamente processadas comercializadas em Campo Mourão, PR. Brazilian Journal of Surgery and Clinical Research 2013; 5(3):17-22.

16. Bruno LM, Queiroz AAM, Andrade APC,Vasconcelos NM, Borges MF. Avaliação microbiológica de hortaliças e frutas minimamente processadas comercializadas em Fortaleza, CE. B. CEPPA 2005; 23(1):75-84.

17. Mogharbel ADI. Validação do emprego de instrumentos de coleta de dados, alface e manipuladores como indicadores de boas práticas em lanchonetes [tese]. Curitiba: Programa de doutorado em Tecnologia de Alimentos de Curitiba; 2007.

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