Biofortification of broccoli seedlings with selenium: influence on bioactive compounds and \textit{in vivo} toxicity

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RESUMO

Biofortificação de mudas de brócolis com selênio: influência nos compostos bioativos e toxicidade in vivo

Os objetivos desse estudo foram avaliar a influência da biofortificação com selênio nos compostos bioativos de brócolis; testar sua toxicidade e mutagenicidade in vivo como forma de comprovar sua segurança para consumo; desenvolver micropartículas pela técnica de spray dryer e avaliar suas propriedades físico-químicas. As mudas de brócolis foram biofortificadas pela adição de 2 mL de solução de 50 µM de selenato de sódio 15 dias após a semeadura (DAS) e coletadas aos 30 DAS. As amostras foram analisadas por diferentes métodos para a quantificação de selênio (ICP-MS, XRF portátil e EDXRF de bancada). O método de fluorescência de Raios X por dispersão em energia feito em equipamentos de bancada ou portátil apresentou desempenho adequado para quantificação de selênio aliado à baixo custo e menor tempo de análise. A técnica de biofortificação com selênio aumentou a concentração desse micronutriente nas mudas de brócolis. Além disso, também proporcionou aumento significativo nos compostos bioativos, tais como, clorofila (12%), compostos fenólicos (26%) e flavonoides (19%), vitamina C (32%) e sulforafano (12%), principal composto bioativo com potencial anticarcinogênico do brócolis. Verificada a eficiência da biofortificação com selênio, conduziu-se estudos de toxicidade e mutagenicidade em camundongos fêmeas e machos tratados com diferentes doses de mudas de brócolis biofortificadas com selênio (15, 45 e 70 µg Se/kg peso corporal). Após 40 dias consecutivos de tratamento, observou-se que os animais tratados com mudas de brócolis com selênio nas doses de 45 e 70 µg Se/kg peso corporal provocaram significativas alterações nos pesos relativos dos órgãos vitais (fígado, baço e rins) e reprodutivos (ovário/testículos), com presença de alterações histológicas nos rins e no baço, em camundongos de ambos os sexos. Nos camundongos machos, a dose de 70 µg Se/kg peso corporal provocou aumento da frequência de eritrócitos policromáticos micronucleados. Nas fêmeas este aumento não foi observado, demonstrando maior sensibilidade dos machos ao tratamento. Por fim, através da técnica de microencapsulação por spray dryer desenvolveu-se micropartículas a base de extratos etanólicos de mudas de brócolis com e sem selênio. As amostras apresentaram baixa umidade (4-5%) e higroscopicidade (11-12 g de água absorvida 100^1 g de micropartícula) e alta solubilidade (99%). No entanto, houve uma redução significativa no teor de compostos fenólicos e atividade antioxidante após o processo. Os resultados desse estudo demonstram a importância da biofortificação do selênio como uma estratégia para aumentar o teor de compostos bioativos no brócolis. Com relação à toxicidade, a dose de 15 µg Se/kg peso corporal não apresentou efeitos tóxicos significativos em machos e fêmeas, porém apresentou potencial mutagênico em machos. Além disso, comprovou-se que a microencapsulação possibilitou a elaboração de amostras com boas características tecnológicas, porém, estudos de otimização devem ser conduzidos a fim de aumentar o poder de retenção dos compostos bioativos e atividade antioxidante.

Palavras-chave: Brassica oleracea; Selenato de sódio; Alimentos funcionais; Mutagenicidade; Micronúcleo; Spray dryer
ABSTRACT

Biofortification of broccoli seedlings with selenium: influence on bioactive compounds and in vivo toxicity

The objective of this study was to evaluate the influence of biofortification with selenium in the bioactive compounds of broccoli; to test their toxicity and mutagenicity in vivo to prove their safety for consumption; to develop microparticles by the spray dryer technique and to evaluate its physicochemical properties. The broccoli seedlings were biofortified through the addition of 2 mL of 50 μM sodium selenate solution 15 days after sowing (DAS), and collected 30 DAS. The samples were analyzed by different methods for the quantification of selenium (ICP-MS, benchtop and handheld EDXRF). The energy-dispersive X-ray fluorescence spectrometry made either in bench or in a portable equipment presented a good performance for the quantification of selenium, together with low cost and less analysis time. The selenium biofortification technique increased the concentration of this micronutrient in broccoli seedlings. In addition, it also allowed a significant increase in bioactive compounds, such as chlorophyll (12%), phenolic (26%) and flavonoid (19%) compounds, vitamin C (32%) and sulforaphane (12%), the main bioactive compound with anticarcinogenic potential. The efficiency of biofortification with selenium in the culture was verified, and toxicity and mutagenicity studies were conducted in female and male mice treated with different doses of selenium-biofortified broccoli seedlings. After 40 consecutive days of treatment, broccoli seedlings with selenium at doses of 45 and 70 μg Se/kg body weight resulted in significant changes in the relative weights of vital (liver, spleen and kidneys) and reproductive organs (ovary/testis), with the presence of histological alterations in the kidneys and spleen in mice of both sexes. In male mice, the dose of 70 μg Se/kg body weight increased the frequency of micronucleated polychromatic erythrocytes. In females this increase was not observed, demonstrating greater sensitivity of males to the treatment. Finally, microparticles of ethanolic extracts of broccoli seedlings with and without selenium were developed through the spray drying microencapsulation technique. The samples presented low moisture (4-5%) and hygroscopicity (11-12 g of water absorbed 100/g of microparticle) and high solubility (99%). However, there was a significant reduction in the content of phenolic compounds and antioxidant activity after the microencapsulation process. The results of this study demonstrate the importance of biofortification of selenium as a strategy to increase the content of bioactive compounds in broccoli and that the dose of 15 μg Se/kg body weight had no significant toxic or mutagenic effects. In addition, microencapsulation has been shown to allow the elaboration of samples with good technological characteristics however, optimization studies should be conducted to increase the retention power of bioactive compounds and their antioxidant activity.

Keywords: Brassica oleracea; Sodium selenate; Functional foods; Mutagenicity; Micronucleus; Spray dryer
1. INTRODUCTION

Fruits and vegetables are the main sources of nutrients and bioactive compounds in the human diet. However, these foods do not always have sufficient or bioavailable quantities of these compounds. Statistics show that around the world, about two billion people, or one in three people, have micronutrient deficiencies (RITCHIE; ROSER, 2018). In this context, agriculture has focused not only on food production to reduce hunger, but also on the production of nutrient-rich food to reduce hidden hunger (KENNEDY; NANTEL; SHETTY, 2003). Thus, to meet the need for some micronutrients and to combat their deficiency some strategies are suggested, such as fortification and biofortification. The fortification refers to the addition of nutrients to food products, for example the addition of iodine to salt. However, despite being an effective strategy, it has some disadvantages/limitations, such as limited stability of the additives, interference of the additives in food quality and the high cost for its realization, requiring advanced infrastructure, which makes its use difficult in developing countries (GÓMEZ-GALERA et al., 2010, RAWAT et al., 2013).

Biofortification, in turn, is a more effective, economical and sustainable method. It allows the synthesis or accumulation of micronutrients in a food crop using conventional and transgenic methods, and can be grown and distributed through existing farming practices. When consumed regularly, biofortified food crops can lead to significant improvements in human health, being a great alternative especially for populations that have limited access to varied diets or other interventions (DÍAZ-GÓMEZ et al., 2017; BOUIS; SALTZMAN, 2017). HarvestPlus and its partners have already proven the effectiveness of this method, with more than 20 million rural households in developing countries having access to biofortified crops. In addition, statistics suggest that by 2030 biofortification could reach up to one billion people (BOUIS; SALTZMAN, 2017).

It is important to note that no single intervention strategy can fully address the problems of micronutrient deficiency and that biofortified foods can not deliver as high levels of vitamins and minerals as food supplements or fortified foods. However, the biofortification strategy acts as a complement to existing interventions, aiding in the daily adequacy of micronutrients intake throughout life. Compared to other intervention methods, biofortification has two main advantages: the ability to reach rural populations and long-term cost-effectiveness. Even though, there is a need for an initial investment - unlike other strategies, which require continuous financial disbursements. From the establishment of the cultivation conditions to the costs of the production, monitoring and maintenance are reduced. In addition,
it is possible for developed crops to be applied in other environments and geographies, increasing the benefits of the initial investment (BOUIS et al., 2011; BOUIS, SALTZMAN, 2017).

In Brazil, through the HarvestPlus Latin American and Caribbean (LAC) program, led by the Brazilian Agricultural Research Corporation (EMBRAPA), BIOFORT Brazil was created. This program has developed and released some nutrient-rich varieties of sweet potato (up to 115 ppm provitamin A), maize (up to 7.5 ppm provitamin A), cassava (up to 9 ppm provitamin A), cowpeas (up to 77 ppm iron and 53 ppm zinc) and beans (up to 80 ppm iron and 50 ppm zinc) (SALTZMAN et al. 2013).

Another micronutrient that has stood out in biofortification research is selenium, an essential micronutrient for humans and animals (DENG et al., 2017). When consumed in small doses it plays important biological functions in the organism (MALAGOLI et al., 2015), which has aroused the interest in this compound in the last three decades (RAYMAN, 2012). Through co-translational mechanisms, selenium is incorporated into proteins as part of the amino acid selenocysteine, the twenty-first essential amino acid (ROMAN; JITARU; BARBANTE, 2014). From this amino acid about 25 selenoproteins are formed, of which one third has the main function of minimizing oxidative damage (KUMAR; PRIYADARSINI, 2014). Scientific studies have already demonstrated the essential role of selenium in various functions in the human organism, such as antioxidant defense (AHMAD et al., 2012), immune function (KHOSO et al., 2015), thyroid hormones formation (WICHMAN et al., 2016), fertility and reproduction (MEHDI et al., 2013). In addition, in the last 20 years, a direct relationship between selenium and cancer has been demonstrated (IBÁÑEZ et al., 2011; JAYAPRAKASH; MARSHALL, 2011; LAMBERTO et al., 2013; MORENO et al., 2012; ROMAN; JITARU; BARBANTE, 2014; BACHIEGA et al., 2016).

Selenium compounds may be present in inorganic (selenate, selenite and selenide) and organic forms (methylselenocysteine, selenomethionine and selenocysteine) (RAYMAN, 2008). These forms are found in different sources, which may present variations in their content due to the amount of selenium present in the soil. Therefore, the same food can present different amounts of this nutrient when cultivated in different areas (MEHDI et al., 2013).

Although the recommended daily dose of selenium is not high, the sources of this micronutrient carry low amounts of it, which makes selenium deficiency a global concern (VALDIGLESIAS et al. 2010; WAEGENEERS et al., 2013). There are reports stating that selenium deficiency affects 800 million people worldwide (MALAGOLI et al., 2015) and, in
European countries, recommended daily intakes are not achieved (IVORY; NICOLETTI, 2017).

As the main sources of selenium, we can highlight plants and meats. In fruits and vegetables, it can vary from 1 to 60 ng g\(^{-1}\) of whole matter, and in cereals this variation is from 20 to 370 ng g\(^{-1}\) of whole matter. In meat, meat products and eggs the content of selenium ranges from 100 to 810 ng g\(^{-1}\) of whole matter. In dairy products it ranges from 10 to 160 ng g\(^{-1}\) of whole matter, and in marine fish from 400 to 1500 ng g\(^{-1}\) of whole matter (KUMAR; PRIYADARSINI, 2014). In Brazil, we have one of the main sources of selenium - the Brazil nut (Bertholletia excelsa HBK), which stands out with contents ranging from 5.8 to 169.9 μg g of whole matter\(^{-1}\) (PACHECO; SCUSSEL, 2007; COMINETTI et al., 2012; ROCHA et al., 2014). However, despite its richness in selenium, this source is still not easily acquired by the Brazilian population (KUMAR; PRIYADARSINI, 2014).

As the vegetables represents the main selenium source for humankind, agricultural crop selenium biofortification becomes even more important as a way to reduce health problems related to this nutrient deficiency (MALAGOLI et al., 2015). Considering this, in 1980 Finland began to perform the genetic improvement of some cultures and to introduce selenium in their fertilization, significantly increasing the selenium levels in the population’s blood (ALFTHAN et al., 2015).

The selenate or selenite are the main inorganic forms of selenium used in the soil to biofortification of plants with selenium. This process can be performed by foliar application, soil application, or the combination of both (ZHU et al., 2017). During this process, accumulation capacity and the form of accumulated selenium will also be different among plants, a fact probably related to the expression levels of sulfate transporters. Due to this variation, some species naturally tend to accumulate greater amounts of selenium, being possible to emphasize the species Allium spp. and Brassicas spp. (HSU et al., 2011; TERRY et al., 2000). Among Brassicas, broccoli is classified as the primary accumulator of this nutrient because of its great capacity to accumulate selenium (＞ 2000 mg kg\(^{-1}\)) (RAMOS, 2011). According to Ávila et al. (2013), inflorescences and sprouts of broccoli accumulate significant amounts of Se-methylselenocysteine.

After the production of Se-biofortified plants, an important step is the quantification of this micronutrient in the food matrix. For this purpose, Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and Energy dispersive X-ray fluorescence (EDXRF) spectroscopy (variant of X-ray fluorescence spectrometry) are the main techniques used. ICP-MS, among the atomic spectrometry techniques, is the most powerful due to its low detection limits. In addition,
it has a rapid detection and ability to measure isotope ratios (POLATAJKO; JAKUBOWSKI, SZPUNAR, 2006; HE et al. 2017). However, this technique requires the complete destruction of the matrix, which increases the time and cost of the analysis. Moreover, the samples digestion with strong acid reagents requires greater caution during sample preparation (TEZOTTO et al. 2013).

On the other hand, EDXRF has shown to be a very promising technique among instrumental analytical methods, being versatile for application in several fields of research, industry and science (VELOSO; SILVA, 2018; ROMANO et al. 2014). This method is based on measuring the energies and fluorescence intensities of each element. X-rays emitted from an ionized atom have characteristic energies for each element, functioning as their fingerprint. Thus, the elements contained in the studied matrix can be identified through the energy of the peaks generated in the spectrum (SILVA et al. 2012; MANSO; CARVALHO, 2007). The main advantages of this technique involve the non-destruction of the sample, the low cost and the short period of analysis (ROMANO et al., 2014), which has encouraged its use in recent years (FLEMING et al., 2015; JOLLY et al., 2017).

As previously mentioned, the biofortification of broccoli with selenium is a great alternative to increase the concentration of this micronutrient in the food. However, in addition to the positive effects mentioned, this process can also promote some negative events (CHOMCHAN; SIRIPONGVUTIKORN; PUTTARAK, 2017) since, in the plant, it can generate an abiotic stress. This stress can significantly alter the amount of bioactive compounds present in broccoli, since the assimilation of selenium can affect the metabolic pathways of sulfur and nitrogen. Changes in the sulfur pathway may directly affect the nitrogen pathway, resulting in alterations in the synthesis of proteins and amino acids such as methionine, phenylalanine, tyrosine and tryptophan, precursors of glucosinolates, and phenylalanine, a precursor of phenolic compounds (MALAGOLI et al, 2015). Thus, the study of the evaluation of the influence of biofortification on bioactive compounds becomes very important, allowing to determine if the process of broccoli enrichment causes the increase or decrease of bioactive compounds.

Another important issue when referring to biofortification is the safety of the consumption of biofortified foods, since the range between selenium benefits and toxicity is narrow. According to the Food and Nutrition Board (FBN) of the Institute of Medicine of the National Academy (USA), the recommended daily intake and the maximum tolerable selenium intake are 55 and 400 μg day$^{-1}$ respectively (OTTEN, HELLWIG, MEYERS, 2006). However, selenium toxicity is not only dependent on the dose, but also on the form and state of oxidation...
The main generalized symptoms of selenium toxicity include skin, mucous and eyes irritated, weight loss and anemia. On the other hand, the most specific symptoms involve garlic odor in respiration and sweat beside irritation in the pharynx, intestine and bronchi (BENKO et al., 2012).

The knowledge that certain agents (physical, biological or chemical) can interact with the genetic material and cause mutations is a long-standing one. The major concern is that these damages result, therefore, in genomic instability and cancer (MALLING, 2004). Therefore, in addition to acute and/or repeated dose toxicity studies, the assessment of the mutagenic potential is a mandatory toxicological evaluation to ensure the safety of the sample. One of the widely used tests for this purpose is the mammalian in vivo micronucleus test, which evaluates the potential of the sample to cause cytogenetic damage, resulting in micronuclei (MN) formation containing either whole chromosomes or lagging chromosome fragments (ARALDI et al., 2015). MNs can be originated by the disruption of the mitotic apparatus (aneugensis) or by chromosomal breaks (clastogenesis) (SAMANTA; DEY, 2012). In adult rodents MNs are formed in the spleen or bone marrow during erythropoiesis. After 6h of final mitosis, erythroblasts originate the polychromatic erythrocytes (PCE), which subsequently undergo a maturation process and give rise to normochromic erythrocytes (NCEs). Thus, the mutagenic agents cause a change in the chromosomes (loss or fragmentation) during the cell division, giving rise to the MNs (KRISHNA; HAYASHI, 2000).

MN is therefore a small fragment of nucleus left behind along the cell division (SYLVIA; BASKARAN; BHAT, 2018). The increase in the frequency of micronucleated PCEs (MNPCES) is indicative of chromosomal damage and has been used as a marker since 1959 (KRISHNA; HAYASHI, 200; KRISHNA; DEY, 2012). The in vivo experiment also allows the evaluation of the absorption, tissue distribution, metabolism and excretion influences on the samples toxicity (MORITA et al., 2016).

Besides the aspects evolved in the biofortified food production and the influence these processes on plant metabolism and safe consumption, another important point concerns how to transfer the benefits of bioactive compounds from these biofortified foods to the consumer market. One of the strategies to carry out this transfer is the production of powders with high nutritional value. Due to their stability and ease storage, these powders can be introduced in different matrices, meeting the requirements of the food industries (RAGHAVI; MOSES; ANANDHARAMAKRISHNAN, 2018; SHISHIR; CHEN, 2017).

Among the technologies already available in the food industry we can mention microencapsulation. This technology has as its primary function the protection of process-
sensitive compounds (DIAS; FERREIRA; BARREIRO, 2015). Developed approximately 60 years ago, microencapsulation refers to the technique of conditioning liquids, solids or gases in which the release of the active content will occur in a controlled manner under specific conditions (DESAI; PARK, 2005).

Spray drying is one of the most successful microencapsulation techniques in the industry. In this process, liquid foods are transformed into powders in a single-step procedure (TONTUL; TOPUZ, 2017), being more economical than lyophilization (eight times) and vacuum drying (four times). In addition, this process has a low drying time (5-100 s), which contributes to a better preservation of heat-sensitive compounds and attributes such as taste, odor and color (SANTIVARANGKNA; KULOZIK; FOERST, 2007; SOSNIK; SEREMETA, 2015). Moreover, the powders produced in this process are resistant to oxidative and microbiological degradation, since they present low levels of water activity (0.2-0.6) and moisture (2-5%) (SHISHIR et al., 2016; PATIL; CHAUHAN; SINGH, 2014; TAN et al., 2011).

In this context, the present study was conducted aiming the production of broccoli seedlings biofortified with selenium. Employing different analytical methods, the broccoli seedlings products were characterized as their selenium and bioactive compounds contents as well as being evaluated for in vivo toxicity and mutagenicity in adition to the development of the microparticles through the spray drying technique.

Thus, this thesis was organized in five chapters. The first is the introduction about the study. In the second chapter, the selenium quantification in the matrix studied was described and using three different methods (benchtop and handheld EDXRF and ICP-MS). In the third chapter, we evaluated the biofortification influence in the bioactive compounds (chlorophyll, phenolic and flavonoid compounds, carotenoids, vitamin C and sulforaphane) profile and quantity from broccoli seedlings. In the fourth chapter, male and female mice were fed broccoli seedlings biofortified with selenium at different doses to assess toxicity through hematological, biochemical and histopathological biomarkers and mutagenicity through the micronucleus test. Finally, the fifth chapter evaluated the possibility of producing microparticles from broccoli seedlings extract by spray dryer and surveyed their physicochemical properties.
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2. BENCHTOP AND HANDHELD ENERGY DISPERSIVE X-RAY FLUORESCENCE (EDXRF) AS ALTERNATIVE FOR SELENIUM DETERMINATION IN BROCCOLI SEEDLING

ABSTRACT

Selenium (Se) has beneficial or toxic effects depending on its concentration, encouraging its determination in food matrices. Inductively coupled plasma mass spectrometry (ICP-MS) is one of the most used techniques due to its high sensitivity. However, due to some disadvantages (high cost, longer analysis time and being destructive) there is a need to search for new alternatives for Se quantification. This study aimed at establishing the instrumental parameters for Se quantification using two energy-dispersive X-ray fluorescence spectrometry (EDXRF) techniques (benchtop and handheld) in Se biofortified broccoli seedlings in comparison to ICP-MS. The results showed that both EDXRF systems and ICP-MS presented similar results for sodium selenate treatments (MC). However, for control (MS) treatments, EDXRF techniques were not able to perform quantification due to the high limit of detection (LOD, 0.6–0.9 mg kg\(^{-1}\)) unlike the ICP-MS (LOD, 0.0007 mg kg\(^{-1}\)). This study demonstrates that EDXRF system are suitable techniques for the determination of Se in biofortified samples.

Keywords: X-ray fluorescence; Complex matrices; Biofortification; Trace elements; Sodium selenate

CONCLUSIONS

The EDXRF systems (benchtop and handheld) presented similar performance compared to the ICP-MS for high Se concentration samples. The choice of the proper filter is an important step in the Se determination by EDXRF. The benchtop and handheld units presented similar Se LOD at 0.6 – 0.9 mg kg\(^{-1}\) range for this matrix with no spectral interference for Se evaluation in broccoli matrix. Although EDXRF approaches are not able to determine Se in samples at either \(\mu\)g kg\(^{-1}\) or sub \(\mu\)g kg\(^{-1}\), both EDXRF systems offered a fast, accurate and lowcost alternative for Se determination in Se-enriched broccoli seedlings and it can be extended to other foods with Se at mg kg\(^{-1}\) range.
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3. CAN BIOFORTIFICATION WITH SELENIUM INCREASE HEALTH-PROMOTING COMPOUNDS IN BROCCOLI (Brassica oleracea L. var. ITÁLICA)?

ABSTRACT

Biofortification of broccoli with Se may alter the profile and amount of bioactive compounds. In this study, the influence of Se treatment (50 µM sodium selenate) on the health-promoting compounds of broccoli was explored. Broccoli seedlings content of 22.43 ± 2.69 µg Se g⁻¹ fresh weight (FW). The treatment significantly increased the antioxidant activity, as well as ascorbic acid (12%), total phenolics (26%) and flavonoids (19%), and chlorophyll (12%) contents. Biofortification with Se increased the content of sulforaphane, main bioactive compound of broccoli, by approximately 12%. Furthermore, there was a significant increase in caffeic, p-coumaric and trans-ferulic acids, and the latter showed moderate activity in the on-line HPLC-ABTS assay, contributing 9.71% to the total antioxidant activity. However, biofortification did not influence carotenoids or chlorophyll b. Biofortification of broccoli with Se is a viable alternative to increase the bioactive compounds in this vegetable.

Keywords: Antioxidant; Brassicas; Bioactive compounds; Functional foods; Sodium selenate; Sulforaphane

CONCLUSION

Our hypothesis was successfully proven that the sodium selenate as inorganic Se was efficient in producing broccoli seedlings Se-biofortified. While the treatment significantly increased the content of health-promoting compounds, such as sulforaphane, chlorophyll (a and total), phenolic compounds, flavonoids and vitamin C, there was no influence on the carotenoid or chlorophyll b contents. Trans-ferulic acid was the only one to present antioxidant activity in the on-line HPLC–DAD–ABTS method. Such results highlight the importance of carrying out studies focusing on biofortification and may help in the investigations of the influence of biofortification with Se in bioactive compounds of broccoli. Se-enriched plants may be an alternative to increase Se levels and to provide significant levels of compounds with health benefits to the human population.
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4. TOXICITY AND MUTAGENICITY OF BROCCOLI SEEDLINGS
BIOFORTIFIED WITH SELENIUM

ABSTRACT

Selenium is a contradictory micronutrient. In low doses, this micronutrient presents benefits to human health; however, in larger doses it can be potentially toxic. The broccoli seedlings (Se-biofortified and control) were evaluated for their toxicity and mutagenicity in male and female mice. Animals were orally treated administered by gavage and the doses of broccoli seedlings with selenium were 15, 45 and 70 µg Se/kg-BW. After 40 days of treatment body weight, viscera index, blood biochemical and hematological parameters and histopathological examination were conducted. The mutagenic potential was analyzed by mouse bone marrow micronucleus assay. The results showed that selenium broccoli seedlings at different doses did not cause significant toxicological alterations in body weight gain and hematological parameters of male and female mice. Biochemical parameters indicate significant changes in males in the UR and AST values in the groups treated with 45 µg Se/kg-BW. Significant alterations were observed in the relative weight of the liver, spleen, kidneys and ovary/testis, and histological changes in the kidney and spleen of male and female mice treated with the highest dose of selenium. After micronucleus counting, a significant increase in micronucleus frequency was observed in male mice at dose of 70 µg Se/kg-BW. From this study, we can conclude that selenium-biofortified seedlings at a dose of 15 µg Se/kg-BW have a high potential for selenium supplementation and do not present obvious toxic effects and the toxic effects were observed in doses higher than that proposed as cancer chemopreventive in humans. However, further studies should be conducted to evaluate the mechanisms of toxicity and mutagenicity of broccoli seedlings biofortification with selenium.

Keywords: Biofortification; Brassica oleracea; Sodium selenate; Micronucleus; Safety

CONCLUSION

This is the first report on the safety assessment of selenium-enriched broccoli seedlings. In summary, investigation into oral and mutagenic toxicity of broccoli seedlings with selenium in different doses were performed in male and female mice. Our results showed no important changes in body weight gain and blood count, but toxic effects were observed in organs such as kidneys, spleen, liver and testis/ovary. In male mice, the frequency of micronuclei increased at dose of 70 µg Se/kg-BW, indicating a possible mutagenic effect. Moreover, these toxic effects were observed in doses higher than that proposed as cancer
chemopreventive in humans. More studies are required to get a deep insight into the toxicity and mutagenic mechanisms of broccoli seedlings biofortified with selenium.

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5. THE SPRAY DRYING TECHNOLOGY INFLUENCE ON THE PHYSICOCHEMICAL PROPERTIES OF SELENIUM-ENRICHED BROCCOLI SEEDLINGS EXTRACTS

ABSTRACT

The search for bioactive compounds-enriched foods grows each year due to its potential health benefits. However, many of these compounds have high instability and sensitivity, requiring special processes to preserve them. This study aimed the production of dried extracts from broccoli seedlings biofortified or not with selenium using the spray drying technology. The extracts were spray dried with 30% of maltodextrin (carrier) at the temperature of 150 °C. The powders were characterized by physicochemical properties (moisture, hygroscopicity, solubility, morphology, total phenolic compounds and antioxidant activities). The microparticles had low values of moisture (4-5%) and hygroscopicity (11-12% g of adsorbed water 100 g-1 of microparticle) and high solubility (98-99%), being therefore biochemically and microbiologically stables. In terms of the morphology, the samples presented a wrinkled surface. The total phenolic compounds and antioxidant activities of the extracts were drastically reduced by the spray drying process and requires further studies to optimize microencapsulation processes.

Keywords: Maltodextrin; Sodium selenite; Microencapsulation; Antioxidant and powder technology

CONCLUSIONS

The microparticles formed from the ethanolic extracts of broccoli seedlings with or without selenium had good physical properties, such as low moisture and hygroscopicity and high solubility. However, the extracts showed a significant reduction of total phenolic compounds and antioxidant activities after the spray drying process. Thus, it is necessary to study the optimization of the microencapsulation parameters to obtain microparticles with a greater potential of bioactive compounds retention.

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