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# Use of products of vegetable origin and waste from hortofruticulture for alternative culture media

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**Abstract**

Conventional culture media are expensive owing to their constituents. Thus, several studies have sought to develop and evaluate the efficacy of alternative, low-cost culture media, in most cases, using natural and easily accessible raw materials. The present study is a literature review, observing various formulations of culture media based on products of plant origin for the growth of microorganisms and production of microbial compounds of industrial interest. In most formulations, vegetable substrates, such as soy, certain beans, corn, and rice, were used in addition to hortofruticultural products. Compared to conventional media, the alternative culture media often present satisfactory results in terms of microbial growth efficiency and production cost.

**Keywords:** alternative culture media; low cost; formulations; food waste.

**Practical Application:** This work provide scientific basis for, and encourage the development and use of alternative culture media.

## 1 Introduction

Ways of reusing food waste have been constantly developed and improved, as food waste reaches alarming levels today (Ramírez et al., 2020). Thus, the development of methodologies for the use of different types of food waste, whether of animal or vegetable origin, has aroused the interest of the scientific community, in order to obtain new products or compounds of interest, or to optimize processes (Navarro-Peraza et al., 2020; Leira et al., 2019; Costa et al., 2020).

Agro-industrial waste has great diversity and has been shown to have potential for application in different areas, such as use as a source of fibers, polyphenols and bioactive compounds in general (Costa et al., 2020; Tagliani et al., 2019), organization and management of fruit and vegetable residues for human and animal consumption (Ramírez et al., 2020) and application in the development of alternative culture media (Cruz et al., 2020).

Culture media provides microorganisms with the necessary nutrients for their growth. Numerous raw materials exist for preparing these media that can be used for microbial growth or for other purposes, such as selective, differential media, and media used to stimulate microbial compound production (Rouf et al., 2017).

In developing countries, the high cost of culture media hampers practical microbiology classes and scientific research in institutions with insufficient financial resources (Uthayasooriyan et al., 2016; Jadhav et al., 2018; Cruz et al., 2020). Thus, some studies have noted that vegetable-based formulations maybe advantageous as alternative (non-commercial) culture media, since these substrates provide several nutrients, including proteins, essential for the growth of microorganisms (Ravimannan & Pathmanathan, 2016). These methods are in development, aiming at improved sustainability using waste products or parts of food plants that are rarely used in human food, such as stems and vegetable peels.

In this review, we present recent studies involving alternative culture media that are formulated with products of plant origin. Thus, the objective is to provide as scientific basis for, and encourage the development and use of, these culture media in educational and research institutions with financial limitations for the acquisition of conventional culture media.

## 2 General view of the use of alternative culture media

Microorganisms depend on favorable conditions for their growth, such as optimal temperature and adequate nutrients. Regarding the latter, the components of some vegetables can provide the necessary microbial nutrition upon their use in culture media. Through assessing scientific publications, alternative culture media could be divided according to their purpose: media aimed at microbial growth and media for production of microbial compounds (Table 1).

## 3 Alternative culture media for microbial growth

In addition to high nutritional value, soy plays an important role in the economy of Brazil as a major worldwide export (Cattelan & Dall'Agnol, 2018). Soy is among the most versatile foods regarding its application in culture media. Several commercial media use soy as the main source of nutrients; therefore, its use in alternative culture media has been studied extensively.

Our group published a study in which textured soy protein (PST), easily acquired in markets, was used in concentrations

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**Table 1**. Products of vegetable origin and waste from hortofruticulture used in the composition of different alternative culture media.

| Food or food residue used | Function | Reference |
|---|---|---|
| Vegetable drumstick seeds and peels, orange peel, potato peel, cauliflower stem and fenugreek stem | Cultivation of different bacteria | Jadhav et al. (2018) |
| Textured soy protein | Cultivation of Gram-positive and Gram-negative bacteria isolated from food | Cruz et al. (2020) |
| Soy flour | Yeast cultivation | Ravimannan & Pathmanathan (2016) |
| Mango pulp residue | Production of bacterial cellulose by *Komagataeibacter xylinus* | García-Sánchez et al. (2019) |
| Biocomposite of discarded fruits and vegetables | Microalgae cultivation | Medeiros et al. (2020) |
| White beans, broad beans, mung beans, peas, chickpeas, black-eyed beans and lentils | Prodigiosin production by *Serratia marcescens* | Mohammed et al. (2020) |
| Rice, cowpea, lentils, peas, chickpeas, soy protein, mung beans | Cultivation of different bacteria and fungi | Shareef (2019) |
| Manioc, sweet potato (purple skin and white skin), purple yam and white yam | Biomass production of *Pleurotus eryngii* | Andrade (2017) |
| Corn maize liquor | Cellulose production by *Gluconacetobacter hansenii* | Costa et al. (2017) |
| Soy protein hydrolyzate | Cultivation of *Pichia kudriavzevii* L9 (post-harvest fruit rot control agent) | Alves et al., 2016 |
| Compost wastes from fruits and vegetables, residues from sugar cane and vinasse | Production of microalgae biomass | Calixto et al. (2016) |
| Cane molasses, millet, russian waters (by-product of oil extraction) | Lipase production by *Candida rugosa* | Freitas et al., 2016 |
| Onion, corn and garlic peel powder | Cultivation of different bacteria and fungi | Berde & Berde, 2015 |
| Soy molasses enriched with calcium carbonate | Development of lactic acid bacteria | Caldeirão et al. (2015) |

from 0.5% to 10% in the preparation of alternative culture media. Thirty-eight bacteria, including major pathogens and food-related spoilage bacteria, such as *Bacillus cereus, Micrococcus luteus, Escherichia coli, Hafnia alvei,* and *Serratia marcescens*, were inoculated into media containing different concentrations of PST. A concentration of 7.5% allowed the growth of all the tested bacterial species. In addition to being effective for microbial growth, the medium's production cost was 86% less than tryptone soy broth and 68% less than tryptone soy agar. The work demonstrated the viability of an easily formulated, low cost, and highly efficient culture medium (Cruz et al., 2020).

In a study carried out in Sri Lanka aiming to evaluate the growth of yeast strains (*Saccharomyces* sp. and *Schizosaccharomyces* sp.), a culture medium based on soy flour was developed. The authors reported that the alternative soy flour medium was more efficient in growing yeast than the conventional culture medium. An additional advantage is the ease of soy flour acquisition by milling soy, which is approximately 50 times cheaper than the commercial medium containing peptone and yeast extract (Ravimannan & Pathmanathan, 2016).

Caldeirão et al. (2015) evaluated the growth of lactic acid bacteria in a culture medium based on soy molasses. The molasses was obtained from defatted soy flour and washed with a hydroalcoholic solution. Thereafter, the solvent was evaporated, and the concentrated solutes formed the molasses. This Brazilian study showed that all the lactic acid bacteria tested could grow in the soy molasses medium; however, the *Bifidobacterium lactis* Bb-12 and *Lactobacillus helveticus* LH-13 strains grew best in this medium, presenting counts greater than $10^8$ CFU/mL.

Natural and processed soy flour, rice, chickpeas, and corn were also used individually as a basis for alternative media in a Sri Lankan study. The authors tested different microorganisms, comparing their growth in both formulated and commercial media, such as nutrient agar for bacteria and potato dextrose agar (PDA) for fungi. The formulated media allowed the tested microorganisms to grow, advocating the use of cheap and easily acquired raw materials, such as rice, chickpeas, corn, and soy, in formulating alternative media for bacteriological and mycological studies (Uthayasooriyan et al., 2016).

In addition to soy, other vegetables have been used in culture media compositions. In a study conducted in Iraq, natural vegetable protein from various grains (rice, lentils, peas, chickpeas, soybeans, cowpeas, and mung beans) was used to formulate alternative media. The author reports that, with the exception of the rice-based medium, all other media proved effective in growing the tested microorganisms, which included bacteria and fungi commonly associated with food spoilage or diseases (*Staphylococcus aureus*, *E. coli*, *B. cereus*, *Pseudomonas aeruginosa*, *Penicillium* sp., and *Aspergillus* sp.). Given the satisfactory results, the author stated that these alternative media could easily replace conventional culture media (Shareef, 2019). In the Philippines, Gabunia et al. (2019) evaluated corn extract as an alternative growth medium for *S. aureus* and *E. coli*. The results revealed that the alternative medium presented the same results as the commercial nutrient agar.

Andrade (2017) described the formulation of alternative culture media for biomass production from the edible mushroom *Pleurotuseryngii*. The media were developed by submerged fermentation using an infusion of cooked Amazonian vegetable substrates, such as manioc, purple-skinned sweet potato, purple yam, and white yam, at a concentration of 20% (v/v), with the addition of 2% glucose. All preparations resulted in satisfactory biomass production, with emphasis on those based on purple-skinned sweet potato and white yam.

Vegetable stems and fruit peels are often discarded or used in compost; however, these organs have a high nutritional value, since fruits and vegetables have a considerable amount of starch and proteins, and their use could considerably reduce the cost of producing culture media (Jadhav et al., 2018).

A study in India used powdered onion, corn, and garlic skins combined in a culture medium called GCO. Bacteria, such as *Bacillus* sp., *Sarcina* sp., *P. aeruginosa*, and the fungi *Candida albicans, Saccharomyces cerevisiae, Penicillium chrysogenum, Aspergillus niger*, and *Trichoderma viridae,* were used to evaluate the efficacy of the GCO medium. According to the authors, the growth of the microorganisms tested using this medium was comparable to that of commercial media, suggesting that this culture medium is economically advantageous for obtaining products from microbial growth (Berde & Berde, 2015).

Jadhav et al. (2018) analyzed the growth of *E. coli*, *Serratia* sp., and *Pseudomonas* sp. in nine formulations containing varying concentrations of drumstick (Moringa) seeds and peel, orange peel, potato peel, cauliflower stem, and fenugreek stem. The results showed that all formulations allowed the growth of the tested bacteria. Additionally, the bacterial growth in three of the nine formulations was greater than that in commercial nutrient broth.

Alternative media can be used to cultivate microalgae, aimed at biomass production for human consumption and aquaculture. The latter has great economic importance, since microalgae form the basis of the marine food chain, serving as a food source for lobsters, shrimp, and oysters (Aversari et al., 2018).

Brazilian researchers evaluated the potential for growth and biomass production of four microalgae species in different alternative media, including media composed of fruit and vegetable waste (prepared from composting fruit and vegetable waste discarded from distribution markets), sugarcane waste products, and vinasse. The results demonstrated that the medium composed of fruit and vegetable waste was promising for microalgae cultivation as it showed higher productivity than those grown in the control media (Calixto et al., 2016).

Medeiros et al. (2020) observed similar results when using an alternative, low-cost medium made with a biocomposite of discarded fruits and vegetables to evaluate the cultivation of microalgae isolated from the Northeast of Brazil. They found that, compared with that in the synthetic culture medium, the cultivation of microalgae in this alternative medium facilitated adequate microalgal growth and improved antioxidant activity and mono and polyunsaturated fatty acid production.

## 4 Alternative media for obtaining microbial products

Although cellulose is commonly produced by plants, some bacteria also produce this polymer, such as those of the genera *Gluconacetobacter*, *Sarcina*, and *Komagataeibacter*. Despite its beneficial applications in industry, the cost of producing bacterial cellulose is very high (García-Sánchez et al., 2019; Costa et al., 2017). In the study by Costa et al. (2017), alternative media formulated with waste products from the food industry, such as sugarcane molasses and corn steeping liquor, were assessed for their efficiency in promoting cellulose production by a strain of *Gluconacetobacter hansenii*. The alternative medium containing cane molasses produced unsatisfactory results. However, the medium with corn steeping liquor provided the highest yield of dry and hydrated bacterial cellulose mass, having an efficiency of approximately 73% of that obtained with the commercial medium.

Recently, a Mexican study investigated the potential of mango pulp as an alternative culture medium for cellulose production by *Komagataei bacterxylinus*. The chemical structure and thermal degradation of bacterial cellulose produced in the mango pulp-based culture medium were comparable with those of cellulose produced in pure sugars. Thus, the authors state that cellulose production in this alternative medium is viable and achieves a high yield. In addition, regarding sustainability, this process takes advantage of an agrifood excess generated in several countries and contributes to the reduction of bacterial cellulose production costs (García-Sánchez et al., 2019).

Lipases are enzymes that catalyze the hydrolysis of triacylglycerol ester linkages, generating free fatty acids and glycerol. These microbial enzymes are widely used in industrial food, organic, and pharmaceutical synthesis. Microbial lipase production in alternative media was described in a study carried out in Portugal. In this work aiming to produce lipases from *Candida rugosa*, the authors used alternative culture media composed of varied concentrations of cane molasses, millets, and Russian water (a byproduct of olive oil extraction). It was observed that the medium containing molasses, millets, and Russian waters in concentrations of 1%, 0.4%, and 0.1%, respectively, produced lipases, suggesting that these products can be used for the production of enzymes, substantially reducing culture media costs (Freitas et al., 2016).

Mohammed et al. (2020) used mixtures of legumes to compose a medium that stimulated the production of prodigiosin pigments in *Serratia marcescens*. The medium was prepared from seven different legume powders: white beans, fava beans, mung beans, peas, chickpeas, black beans, and lentils. The solid and liquid forms of the alternative culture medium stimulated pigment production during incubation at 25-28 °C for 24 h. The authors also tested media of the seven legumes individually to verify the pigment production; however, they observed a decrease in prodigiosin production, with the colonies becoming light red in color. The medium prepared from the combination of legumes proved to be easy to prepare and store. In addition, it was highly effective in stimulating the production of *S. marcescens* pigments, with the advantage that its constituents are available at a low cost.

## 5 Conclusion

In view of various scientific experiments, developing effective and low-cost culture media is a possibility. Many of these media use food items or parts thereof that are not used for human consumption, making them accessible to institutions within sufficient resources for commercial culture media. In addition to promoting accessibility to knowledge, the development of alternative culture media contributes to sustainability by using food waste. Most studies highlight the effectiveness of alternative culture media, indicating similar applicability and, in some cases, superior performance as compared to the traditional culture media. Thus, studies evaluating unexplored food and food waste in this way are promising.

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