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# Ultrasound processing to enhance the functionality of plant-based beverages and proteins

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This opinion paper discusses how ultrasound can be used to modify the structure of plant-based beverages (juices and 'milk') and proteins, achieving new functionalities. Both positive (such as increasing the nutrient and bioactive compound bioaccessibility, improving protein solubility, and modifying its digestibility) and negative (such as degradation of nutrient and bioactive compounds by exposing them to the environment, or the limited microbial inactivation) aspects are discussed. It is clear that ultrasound technology can be used as a valuable tool to improve plant-based beverage properties, helping to achieve clean label products and positively impacting well-being. Its scale-up to industry, however, is still a concern that needs both scientific studies and technological development.

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## Introduction and contextualization

Fruits and vegetables are important parts of the human diet as sources of nutrients and bioactive compounds, such as carotenoids, phenolics (or polyphenols), flavonoids, and vitamins and minerals, being juices, a practical and relevant way to ingest those plant components. Furthermore, there is a rising demand for plant-based products (such as milk analogs) and ingredients (such as proteins). The expansion of the plant-based market is a worldwide trend and is related to several reasons, such as the growth of the vegan, vegetarian and 'flexi-vegans' public, the concern with the environmental impact of food production, sustainability, and animal welfare, in addition to the health aspect (e.g. allergenicity of milk proteins).

Therefore, this paper describes the current research on using ultrasound processing to enhance the functionality of plant-based beverages, considering the three most representative examples in the context: (i) fruit and vegetable juices, produced from top-down (i.e. by disassembling the plant organism toward a smaller scale); (ii) plant-based 'milk', produced from top-down or bottom-up (i.e. by assembling a new product from ingredients), then using (iii) vegetable proteins. In those products, the main objectives of using ultrasound are preserving nutrients and bioactive compounds, increasing their digestibility and accessibility to the human body, improving physical stability, rheology, and interaction with water, thus achieving desirable sensorial impact, better healthy aspects, and promoting well-being.

More information about using ultrasound technology in food products, ingredients, and processing can be obtained in the recent review articles of [1–6].

## Ultrasound processing of fruit and vegetable juices

Fruit and vegetable juices are particulate solid–liquid systems, constituted by a suspension of cells and their fragments, including fibers and cell walls, in the serum (water + soluble material). Ultrasound processing modifies this structure in different stages, as described by Rojas, Leite, Cristianini, Alvim, and Augusto [7], impacting their physical, chemical, sensorial, and nutritional modifications — as recently detailed by Rojas, Kubo, Caetano-Silva, and Augusto [4••]. Table 1 presents recent studies in ultrasound-processed juices, where the main results are discussed.

The cell disruption and reductions in particle size promote new particle–particle and particle–serum interactions that allow increasing of cloudiness, suspension stability, and

**Table 1**

**Ultrasound processing and main reported impact on fruit and vegetable juices, regarding physical modifications, compound content, and quality:** Increase or Improvement, Decrease or Negative effect, **and** No variation, **compared with untreated or fresh juices.**[13••–15,19,21,22,24–26,28]

| Juice source | Processing conditions | Main results of ultrasound processing | |
|---|---|---|---|
| Spinach [8•] | TS [30 kHz; 60 °C; 20 min; 200-600 W; 50% dc] Vs [100 mL] | Physical stability, rheological properties, bioactive compounds | Particle size |
| Apple [9] | TS [30-70 °C; 5-12 min; 525-1125 W] Vs [200 mL] | Colour, cloudiness, Physical stability, rheological properties | Particle size |
| Kiwifruit [10] | US [20 kHz; ~ 4 °C; 4-16 min; 400 W; 50% dc] Vs [100 mL] | Colour, cloudiness, rheological properties | Pectin |
| Carrot [11] | US [20 kHz; 30 °C; 10 min; 2s on-2s off; 221 - 321 W] Vs [25 mL] | Apparent viscosity | Colour, carotenoids |
| Plum [12] | TS [40 kHz; 40 - 60 °C; 5 - 30 min; 2s on-2s off; 0.348 W/cm$^3$] | Colour, cloudiness, flavonoids, phenolics, carotenoids, ascorbic acid | Compounds degradation at T > 60°C |
| Tomato [13•] | TS [20 kHz; 37 - 52 °C; 2 - 10 min; 28 - 40 W/cm$^2$] Vs [150 mL] | Lycopene | Ascorbic acid, Lycopene |
| Sweet potato [14] | TS [26 kHz; 8 min; 0.66 W/cm$^2$] Vs [150 mL] | Bioaccessibiity of β-carotene | Colour |
| Guava [15] | US [20 kHz; 25 °C; 0 - 9 min; 15 W/cm$^2$] Vs [200 mL] | Physical stability, bioaccessibility of lycopene | Lycopene |
| Strawberry [16] | TS [40 kHz; 25-50 °C; 5 -15 min; 110 W] Vs [50 mL] | Ascorbic acid, anthocyanin | pH, °Brix, titrable acidity |
| Orange [17] | US [20 kHz; 10 min; ~ 0 °C; 300-1200 W] Vs [10 mL] | Ascorbic acid | Xylooligosaccharides |
| Mango [18] | US [20 kHz; ~ 0 °C; 0-40 min; 5 s on - 5s off; 0-600 W] | Consistency and flow behaviour index | Carotenoid, phenolics |

**Table 1** (*continued*)

| | | | | |
|---|---|---|---|---|
| | Vs [100 mL] | | | |
| Mandarin [19] | US [19 kHz; 50 °C; 36 min; 750 W] | Colour | | Sugar, acid components |
| Orange [20]. | US [19 kHz; 37 °C; 15-150 s; 15-86 J/mL] Vs [30 mL] | Cloudiness | | Ascorbic acid |
| Watermelon [21] | US [26 kHz; 4-16 min; 80 W; 50%A] Vs [100 mL] | Lycopene, phenolics, flavonoids, antioxidant capacity | Ascorbic acid | pH, °Brix, titrable acidity |
| Açaí (*Euterpe precatoria*), buriti (*Mauritia flexuosa*) [22] | US [20 kHz; 70 °C; 10 min; 0.9-3.6 kJ/cm$^3$] Vs [100 mL] | Fatty acids, Bioaccessibility and concentration of anthocyanins, carotenoids | Fatty acids, Colour | pH, °Brix, titrable acidity |
| Açai [23•] | US [19 kHz; 25 °C; 2-10 min; 75.34-272.93 W/cm$^2$] Vs [150 mL] | Sugar, betaine, bioaccessibiity of ascorbic acid. | | |
| Orange [24] | TS [28 kHz; 10-50 °C; 5-25 min; 180-900 W/L] | Aroma, glycosidically bound volatiles | | |
| *Berberis amurensis* Rupr. [25] | US [20 kHz; 10 min; 140 W; 70%A] | Colour, anthocyanins | | |
| Red pitaya [26] | TS [56 °C; 20 min; 475 W] US [10 °C; 20 min; 475 W] Vs [60 mL] | | Colour, betacyanins | |
| Strawberry [27] | TS [24 kHz; 23.5-56.5 °C; 3 min; 0.29 W/mL] | | | Anthocyanins, soluble solids, phenolics |
| Sugar cane [28] | TS [20 kHz; 80 °C; 23 min; 750 W] | | | Phenolics, colour |

TS = thermosonication-processing conditions; US = ultrasound-processing conditions. Regarding processing conditions, it is presented: the frequency, temperature, time, power, or acoustic density or acoustic intensity, duty cycle (dc), and amplitude (A). Vs = volume of sample.

variation on rheological properties [8–11,18]. The rheological properties can be changed temporally or permanently, depending on the ultrasound energy applied, processing time, pulp content, and temperature [7,29]. Therefore, a different impact on the rheological properties is expected, and the processing conditions must be evaluated, depending on the defined target.

On the other hand, color variations are reported, which are related to the decrease in particle size, extraction, and/or degradation of main pigments. In fact, once the cell wall is impacted, the intracellular content is exposed and some compounds become more assessable (to the human body, as a positive aspect, or to oxygen and the environment, as a drawback). The variation in the content of the compound (Table 1) is a balance of improved extraction (causing an apparent increase in the quantified concentration) and exposition to deteriorating conditions (decreasing their concentration). In fact, ultrasound can modify the juice structure with potential health benefits, which can be particularly relevant by considering food products for special needs (Rojas et al., 2021) [4••].

Even so, some ambiguous results are reported, such as for lycopene and ascorbic acid, once they can be easily degraded due to exposure to heat, light, and oxygen. The decrease in such compounds is due to long times, high temperatures, and/or powers of process, while at inverse conditions, increase or no variation occur. In either case, despite the decrease in some compounds, ultrasonic processing is more effective than conventional thermal processing regarding compound preservation [12,16,20,23•] — although this comparison is always difficult, being necessary an evaluation of process target and conditions.

Moreover, ultrasound alone is rarely effective for the required microbial inactivation in juices, even considering that microbial resistance to ultrasound is small at low pH [30]. Therefore, ultrasound has been combined with other technologies to improve its microbial inactivation, such as natural antimicrobials [11,31,32], pulsed light ([33], ozone [34], heat under pressure [35], and, especially, the combination with a thermal process (thermosonication) at mild temperatures) [9,27]. In fact, ultrasound can turn the microorganisms more sensitive to thermal inactivation, such as during the thermosonication to inactivate *Neosartorya fischeri* ascospores [36] and *Saccharomyces cerevisiae* [37] in apple juice, or *Escherichia coli* in apple cider [38]. Therefore, from a microbiological perspective, the use of ultrasound in combination with mild heating or other technologies for industrial use is promissory, although the impact on juice compounds must be evaluated since the combination of unsuitable conditions can be detrimental to sensitive compounds.

Summarizing, ultrasound can improve the functionality of fruit and vegetable juices by improving the stability, physical properties, and bioaccessibility of some bioactive compounds. In addition, it preserves the thermolabile compounds or those added to the juices to increase functionality [17]. However, some aspects should be improved in future studies such as the calculation of the actual ultrasound-power density in the products, the control of the process temperature, evaluation of the stability throughout the storage, and studies of bioaccessibility, bioavailability, and bioactivity.

## Ultrasound processing of vegetable alternatives to milk

There is a rising trend in producing vegetable beverages to be similar to animal milk, concerning the main sensorial and/or nutritional properties. Those beverages, often called plant-based 'milks' (and here referenced as that, to avoid confusions with other plant-based beverages, such as juices, nectars, soft drinks, etc.), are suspensions and emulsions from sources such as soybean, rice, almond, peanut, and oat, among others — thus being physical stability an important challenge.

Ultrasound has demonstrated being useful for improving the physical stability of almond 'milk' using 5 min–300 W [39], coconut 'milk' using 13 min–55 W/cm$^2$ [40•], and peanut 'milk' using 3 min–400 W [41•], which can be related to changes on particle size (proteins and fats) and rheology.

These beverages are reported to present bioactive compounds such as isoflavones, phytosterol and great antioxidant activity [42]. However, plant-based 'milk' can present antinutritional factors such as phytates, saponins, and protease inhibitors [43]. In fact, ultrasound can positively impact the nutritional and bioactive properties of plant-based 'milk'. For instance, it increased the protein digestibility by 7.4%, reducing the trypsin inhibitor by 52% in soybean milk — 400 W, 25 kHz, 16 min [44]. However, no significant improvement was found for almond milk using ultrasound (400 W, 20 kHz, 16 min) [45]. Moreover, ultrasound (400 W, 24 kHz) treatment for 20 min applied to hydrated soybeans produced 'milk' with 63–84% higher isoflavone content than untreated beans [46].

Different works report that ultrasound alone is rarely effective for the required microbial inactivation in plant-based 'milk'. For instance, a reduction of 0.9 log (total plate count) was obtained using a probe of 400 W for 3 min on peanut 'milk' [41•], while a reduction up to 1.3 log was found for *Escherichia coli* and *Listeria monocytogenes* when ultrasound (130 W, 20 kHz) with 80% of power and 8 min of treatment was applied to almond 'milk' [47]. On the other hand, some works report

**Table 2**

**Examples of recent studies on the modification of plant proteins by ultrasound processing and the respective improvements in technofunctional properties: increasing in S: solubility; E: emulsifying; G: gelling; OA: oil-absorption capacity; WH: water holding capacity; F: foaming.[63–66]**

| Type of protein | Improved properties | | | | | | References |
|---|---|---|---|---|---|---|---|
| | S | E | G | OA | WH | F | |
| black gram protein | | | | ✓ | | ✓ | [63] |
| guamuchil seed protein isolate | ✓ | ✓ | ✓ | ✓ | | ✓ | [59•] |
| hemp seed protein isolate | | ✓ | | ✓ | | ✓ | [61] |
| lupin protein isolate | ✓ | | ✓ | | | | [64] |
| *Moringa oleifera* seed protein | ✓ | ✓ | | | | ✓ | [62] |
| pea protein concentrate | | | | ✓ | ✓ | | [65] |
| perilla seed protein isolate | ✓ | ✓ | | ✓ | ✓ | ✓ | [58] |
| potato proteins | ✓ | ✓ | | | | ✓ | [60] |
| potato protein isolates | | ✓ | ✓ | | | | [66] |
| pumpkin-seed protein isolates | | | | | | ✓ | [57] |

microbial inactivation similar to pasteurization processes, which can be an alternative for this product commercialization under refrigeration. For example, Campaniello, Bevilacqua, Speranza, Sinigaglia, and Corbo [48•] reported a reduction on *Salmonella enterica* load up to 8 log in rice 'milk' — although samples experienced up to 48ºC/10 min during processing, being thus relevant the contribution of thermal energy. However, by being low-acid products (pH close to neutrality), the main target of vegetable 'milk' are bacteria spores. Therefore, further studies are needed considering the safety and stability aspects of microbiology, with a focus on specific microbial targets and a combination of technologies.

Summarizing, ultrasound technology has been demonstrated to be beneficial to process plant-based milk obtaining beverages with physical stability during storage and with some nutritional enhancements. The most used device was the ultrasonic probe of different nominal power (130–400 W) and frequency (20–25 kHz), reporting different parameter effects as power-percentage application (20–100%), time (1–60 min), amplitude (50–100%), and temperature (25–60°C). In contrast, ultrasonic bath has not been used in the last 5 years for plant-based milk processing. This probably occurred due to ultrasonic probe being more powerful than the ultrasonic bath, leading to used shorter processing time. Similarly to other products, more studies of processing equipment and conditions, focusing on scale-up, are needed. Particularly, the potential of ultrasound to improve stability, physical, sensorial, and nutritional properties of vegetable 'milk' is just starting to be revealed, and further studies are needed involving different sources, processing approaches and targets, the effect in other antinutritional factors such as saponins and phytates, and how ultrasound treatment affects further processes for elaborating analogs of cheese, yoghurt, or ice cream.

## Ultrasound processing of plant proteins

Proteins are complex biopolymers made up of amino acids and are of great nutritional, sensory, and functional importance. They present different functional properties, depending on factors such as their source, amino acid composition and sequence, molecular weight, structure, conformation, and surface hydrophobicity [49]. Among the various techno-functional properties of proteins, one can highlight the properties related to solubility, thickening, water/oil holding capacity, emulsification, foaming, and gelation. The variety of properties makes proteins important components in the formulation of food products, either as constituents from raw materials (e.g. soy proteins in soy 'milk') or as ingredients (protein concentrates or isolates). However, vegetable proteins might present some limitations due to the unsuitability of their properties for specific applications and formulations in the food industry. To overcome these limitations, chemical, biological, and physical methods have been employed for protein modification, including enzymatic hydrolysis, fermentation, heat treatment, high-pressure processing, and ultrasound [50–53]. Comprehensive reviews on the different approaches for the modification of plant-based proteins can be found in the literature [54,55••].

Recently, the use of ultrasound has attracted attention to modify, improve, and modulate the functional properties of protein concentrates and isolates from different plant sources. Several studies have reported that the application of high-intensity ultrasound to dispersed/suspended proteins resulted in structural changes and thus, modifications of the functional properties of these proteins. Some recent studies are presented in Table 2. These changes are attributed to cavitation, shear stress, turbulence effects, and localized temperature rise, which can promote modification even at a molecular level [6,56–58].

For example, Flores-Jiménez, Ulloa, Urías-Silvas, Ramírez-Ramírez, Bautista-Rosales, and Gutiérrez-Leyva [59•] found that ultrasound altered the secondary and tertiary structures of guamuchil-seed protein isolates, which increased their surface hydrophobicity and molecular flexibility. These structural changes led to a significant improvement of solubility, oil-absorption capacity, emulsification, foaming, and gelation properties. Similarly, Hussain, Qayum, Zhang, Hao, Liu, Wang, Hussain, and Li [60] investigated the effect of high-intensity ultrasound on the bioactive, functional, and structural properties of potato protein and reported that emulsifying ability index, emulsifying stability, foaming ability, solubility, and digestibility were significantly enhanced.

However, it is worth mentioning that depending on the process parameters used, different results can be obtained, and may result in an increase or decrease in protein functionality. In general, if conditions are too intense, especially long sonication times and very high intensities, the (over-)processing can result in an excessive molecular aggregation, leading to the obtention of proteins with poor functional properties [61]. For example, Karabulut and Yemiş [61] observed an increase in solubility of hemp-seed protein isolates after ultrasound processing at the range of 45–65% amplitude (6–11 W/cm$^2$, 27–32 W/cm$^2$) for 5–10 min, which was attributed to the partial unfolding of protein molecules and greater protein–water interaction. However, at higher amplitudes and longer times, the protein solubility was reduced, probably due to the formation of aggregates with higher molecular weights. Similarly, Tang, Du, and Fu [62] observed that solubility, foaming properties, and emulsifying properties of *Moringa oleifera* seed protein increased or decreased, depending on the ultrasonic power. Therefore, to achieve the desired level of functionality, the operating parameters must be chosen/optimized.

In summary, ultrasound processing is an interesting and promising approach for protein modification, which may allow the utilization of a wider variety of vegetable proteins and the expansion of their applications in the food industry. Nevertheless, it should be noted that the focus has been on using sonication for modification of isolated proteins (in suspension), but the modification of proteins in complex food matrices and the potential interaction of proteins with other components should not be neglected.

Future developments are expected to demonstrate the increase of functionality of plant proteins of different sources, for different purposes (including as ingredients for other food products and improvement of nutritional properties of emerging sources).

## Conclusions and perspectives

Ultrasound processing induces a sequence of structural modifications in plant-based products, impacting their properties. The modification involved different steps from the intact tissue to cell and macromolecule spatial changes to disruption. Consequently, different properties are altered, positively or negatively, such as improving the health benefits or sensorial perception, but also degrading compounds of interest due to exposition to detrimental conditions. Therefore, more studies are needed to describe how processing conditions can affect the plant-beverage functionalities — considering different vegetable sources and food products for different needs.

In particular, a persistent challenge is scaling up the ultrasound processing at industrial levels — in special considering that most of the studies apply the probe reactor. Studies of reactor engineering are needed to provide equipment able to process the volumes of the food industry, as well as studies in material science, are needed to develop more resistant probe tips (which wear out by erosion).

We consider the ultrasound technology can be used as a valuable tool to improve the properties of plant-based beverages, helping to achieve clean label products and positively impacting well-being. Its industrial application, though, is close to be effective, although depending on the next scientific studies and technological developments.

## Author contributions

**Meliza Lindsay Rojas:** Conceptualization, Data curation, Formal analysis, Investigation, Project administration, Visualization, Writing – original draft, Writing – review & editing. **Mirian T.K. Kubo:** Conceptualization, Data curation, Formal analysis, Investigation, Project administration, Visualization, Writing – original draft, Writing – review & editing. **Alberto Claudio Miano:** Conceptualization, Data curation, Formal analysis, Investigation, Project administration, Visualization, Writing – original draft, Writing – review & editing. **Pedro E.D. Augusto:** Conceptualization, Data curation, Formal analysis, Investigation, Project administration, Visualization, Writing – original draft, Writing – review & editing.

## Conflict of interest statement

We declare no conflict of interest.

## Data availability

No data were used for the research described in the article.

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