REVIEW

Possibilities of Using High Intensity Ultrasound Technology with Stevia – a Review

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Summary

The application of high intensity ultrasound as an effective non-thermal, inexpensive and simple method in technological processes of food processing and in the food industry has gained great popularity in recent years. High intensity ultrasound is technique based on the postulates of minimal food processing with the main objective of food preserving and reduced degradation rates of biologically active compounds with significant antioxidant activity. High intensity ultrasound shows its wide application in the food and processing industry. In this review article it is described in terms of its application in the foods decontamination process, as a possible replacement of conventional heat treatment by pasteurization for the principal purpose of bioactive compounds (for example phenolic compounds) reduced degradation and as one of the most effective method for extraction of different diterpenic compounds (for example stevioside), aromas, pigments, etc. Stevia and its diterpenic glycosides are popularized as a natural sweetener and as such found their application in preparation of numerous products for food and baking industry. The main challenge in the production of natural sweetener from the stevia leaves is an extraction of stevioside and rebaudioside A with the main objective to increase its efficiency and yield during the process.

Key words: *Stevia rebudiana Bertoni, ultrasound technique, stevioside, phenolic compounds*

Sažetak

Primjena ultrazvuka visokog intenziteta kao efikasne ne termalne, jeftine i jednostavne metode u tehnologijskim procesima prerade namirnica i u prehrambenoj industriji, stekla je veliku popularnost posljednjih nekoliko godina. Ultrazvuk visokog intenziteta tehnika je koja se temelji na postulatima minimalnog procesiranja hrane s glavnim ciljem očuvanja namirnice i smanjene stope degradacije biološki aktivnih spojeva značajne antioksidacijske aktivnosti. Ultrazvuk visokog intenziteta ima široku primjenu u prehrambenoj i prerađivačkoj industriji. U ovom preglednom članku opisan je s aspekta primjene u procesu dekontaminacije namirnice, kao moguća zamjena konvencionalne toplinske obrade procesom pasterizacije, sa svrhom smanjene degradacije bioaktivnih spojeva (na primjer fenolnih spojeva) i kao jedna od najučinkovitijih metoda ekstrakcije različitih diterpenskih spojeva (na primjer steviozida), aroma, pigmenata itd. Stevija i njeni diterpenski glikozidi popularizirani su kao prirodni zaslađivač i kao takvi svoju primjenu pronalaze u pripremi brojnih proizvoda u prehrambenoj i pekarskoj industriji. Glavni izazov u proizvodnji prirodnih zaslađivača iz lišća stevije upravo je ekstrakcija steviozida i rebaudiozida A sa svrhom veće efikasnosti i prinosa.

Ključne riječi: *Stevia rebudiana Bertoni,ultrazvučna tehnika, steviozid, fenolni spojevi*

INTRODUCTION

Phenolic compounds as a secondary metabolites of plants are typical for many vegetable and fruit varieties, and from which, in rich phenolic composition, can be emphasize apples and apple products in general (Markowski and Płocharski, 2006). A group of phenolic compounds in apples is mainly presented by flavonoids and phenolic acids (Markowski and Płocharski, 2006; Scalbert et al, 2005; Manach et al, 2004; Schieber et al, 2001). The beneficial effects of apples on human health is being attributed to its rich content of polyphenolic compounds, of which are even several thousands identified in apple fruit (Sies, 2010), which give a significant antioxidant activity to an apple (Carbone et al, 2011). A thin layer just below the skin of apple fruit contain the highest concentration of phenolic compounds (Ceymann et al, 2012; Hui et al, 2006; Oszmiański and Lee, 1994; Thielen et al, 2005, Awad et al, 2000), and for this reason in the production of the different apple products such as apple juice, fillings, jams, etc., is not advisable to remove the skin. Production technology of fruit fillings assumes the application of two basic technological processes: thermal treatment of foods and correction of flavors with process of sweetening (Sinha et al, 2012). Different methods applied during the processing of raw apple material are the process of pasteurization and heat treatment, grinding of apple fruit to a pulp and long storage period and they significantly influences on the reduction of phenolic compounds (Carbone et al, 2011). Therefore, it is a very important from the processing aspect, to develop the methods of minimal food processing which assumes the use of lower, non-invasive temperature which significantly contributes to the maintenance of bioactive compounds with high antioxidant capacity such as phenolic compounds, but also significant energy savings during implementation in manufacturing processes (Ohlsson and Bengtsson, 2000). One of such minimal food processing methods is

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high intensity ultrasound in this case intended for the purpose of preserving food.

As previously mentioned, during the production of fruit fillings a correction of taste with sweetening process is required. The most commonly used sweeteners in the industry are natural sugar or artificial sweeteners (Sinha et al, 2012), for which is on the basis of different medical researches identified significant harm for human health, from the appearance of diabetes and obesity (consumption of overly high levels of sucrose) (Burke et al, 2003) to the emergence of degenerative diseases, such as brain cancer (use of artificial sweeteners such as saccharin and aspartame) (Jae-Yong et al, 2011).

Besides for the purposes of food decontamination (sterilization and pasteurization processes) in the food industry, high intensity ultrasound has found its wide application as a method of extraction, enhancement of emulsification, in the homogenization, crystallization, as a pretreatment for process of dehydration etc (Brnčić et al, 2009).

Figure 1. *Determination of unwanted foreign bodies using low intensity ultrasound (Brnčić et al, 2009)*

The use of high intensity ultrasound for the purpose of conversion with the conventional thermal process of food by pasteurization

During the application of ultrasound as a method primarily intended for the food industry, it is important to distinguish the ultrasound of low and high intensity. Specifically, low-intensity ultrasound (diagnostic) is only applied as an analytical method since there is no sufficient energy dispersed into medium under high frequencies used with low intensity ultrasound. This technique is used for characterization of foodstuffs, determination of unwanted foreign bodies, determination of liquids level in tanks and velocity of liquids as well. In figure 1 experimental setup for unwanted foreign bodies determination is presented (Brnčić et al, 2009)

High intensity ultrasound finds its application in the food processing and general in the food industry and biotechnology (Brnčić et al, 2009). The main action mechanism of high intensity ultrasound as a method that is applied for the purpose of products decontamination respectively to remove microorganisms and impurity of foods refers to the appearance of cavitation bubbles collapse, causing unsymmetrical fluid pressure near the foods surface (figure 2).

Such unsymmetrical surface disables a breakthrough of liquid, so fluid jet occurs as a result of the main flow on the other side of bubble. As described above, appears a strong jet of fluid which then eliminated impurity and bacteria from the food surface. Another advantage of high intensity ultrasound is that its effect of longitudinal waves can reach cracks of some foods that conventional processing methods such as the pasteurization or sterilization process can not reach (Boucher, 1980). Different scientific researches has shown that high intensity ultrasound can be identified as a potential technology

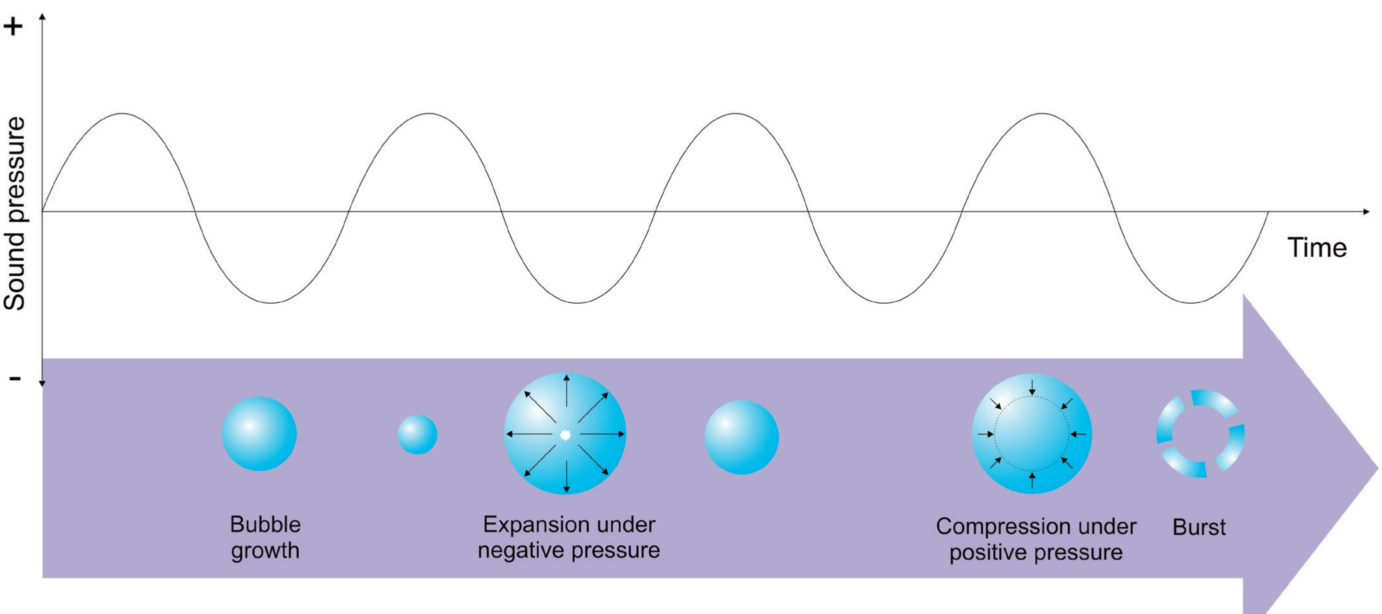

Figure 2. *Principle of cavitation action in liquid medium (Brnčić et al, 2010)*

to remove microorganisms from the food up to the border of the 5-log by its regulations prescribed by FDA commission (Salleh-Mack and Roberts, 2007). The use of high intensity ultrasound technology shows a number of advantages in its application as a substitute for thermal process. Primarily, this refers to the significant reduction of the application duration in the food processing and generally contribution to significant energy savings (Mason et al, 1996; Piyasena et al, 2003, Patist and Bates, 2008). Therefore, in general we can say that the high intensity ultrasound is non thermal method of food preservation that shows great advantage in inactivation of microorganisms in food without causing the side effects (degradation of antioxidant compounds) that are associated with conventional heat treatments. It is important to emphasize that high intensity ultrasound as a non thermal food processing method is not fully effective in the reducing of undesirable microorganisms, especially of high resistance microbial populations. For this reason high intensity ultrasound can not be used as a complete replacement for the process of sterilization. However, the application of ultrasound in combination with pressure and/or heat showed significantly better results on the lethal effect of bacterial cells, spores and yeast. It is 6 to 30 times higher than heat treatment that is carried out at the same temperature (Ross et al, 2003). There are several possibilities of combining high intensity ultrasound with pressure and heat, which are defined in terms of thermosonification (combination of heat and ultrasound), manosonification (combination of pressure and ultrasound) and manothermosonification (combination of pressure, heat and ultrasound), and which have proved to be a very effective treatments in the inactivation of most pathogenic microorganisms and to inactivate the enzymes resistant to ultrasound (Pagan et al, 1999; Knorr et al, 2004.; Bermudez-Aquire et al, 2009; Jeličić et al, 2012). During the application of the above mentioned procedures, very important factor is selection of proper temperature and pressure value which will be combined with process of high intensity ultrasound, primarily because researches shows that their effect is not necessarily synergistic and positive in the case of boosting the effect of ultrasonic cavitation (Raso et al, 1998, Piyasena et al, 2003; Herceg et al, 2009).

The possibilities of higher extraction potential of stevioside using high intensity ultrasound

Stevia rebaudiana Bertoni (figure 3) is plant native from northeastern Paraguay (Dossier, 1999), and today it is cultivated around the world primarily due to its use as a natural sweetener (Špicnagel et al, 2011). Stevia is specific because of its sweetening properties with zero calories (Kroyer, 2009; Seema, 2010), with proven non-toxic effects on human health (Pól et al, 2007), recently found widespread use in the food and baking industry. Steviol glycosides which are concentrated in the plant leaves are even 300 times sweeter than sucrose (Alaam, 2007), the heat-stable (for temperatures about 200°C) and well tolerated to a low pH-values (Abou-Arab et al, 2010). All of these are characteristics that popularize this plant in its application as a natural sweetener in consummation and everyday use.

Figure 3. *Stevia rebaudiana Bertoni leaves (Lemus-Mondaca et al, 2012)*

High intensity ultrasound found its important application in the catalysis of the compounds extraction from organic materials. Classical techniques of compounds extraction from plant materials are based on the correct choice of solvents, together with the use of heat and/or agitation. Extraction of organic compounds contained within the plant tissue and seeds using a solvent could be significantly enhanced by the use of ultrasound energy. Mechanical effects of ultrasound will provide greater penetration of solvent into cellular materials and substantially improve the mass transfer of compounds that dissolve in the solvent. The ultrasound energy alone will enable the destruction of the plant cell walls, and thus facilitate the release of cell contents into the solvent (Mason et al, 1996). The application of high intensity ultrasound has proven to be extremely effective in the extraction of various types of compounds independent of their chemical structure: the proteins, solid materials, secondary plant metabolites (phenolic compounds, glycosides) etc. (Brnčić et al, 2009; Pingret et al, 2012; Alupului et al, 2009).

Stevioside is diterpenic glycoside isolated from the leaves of *Stevia rebaudiana* Bertoni. Above mentioned diterpenic glycoside is specific for its extreme sweetness, even 300 times sweeter than sucrose (Alaam, 2007) without any caloric values, his glycemic index is zero (Puri et al, 2011; Seema, 2010; Kroyer, 2009). Stevioside molecule was built from two basic parts (three dimensional): a carbohydrate residue, 3 glucose molecules linked to diterpenic carboxyl alcohol, aglycone steviol (Puri et al, 2011) (figure 4).

The leaves of stevia which are used as a raw material for production of food additive and sweetener in food products, on the market are present as a green powder obtained by grinding the dried green leaves (Mishra et al, 2010), as a white powder that is obtained by depigmentation process of green powder (Brandle and Rosa, 1992) and as a solution of stevia leaves

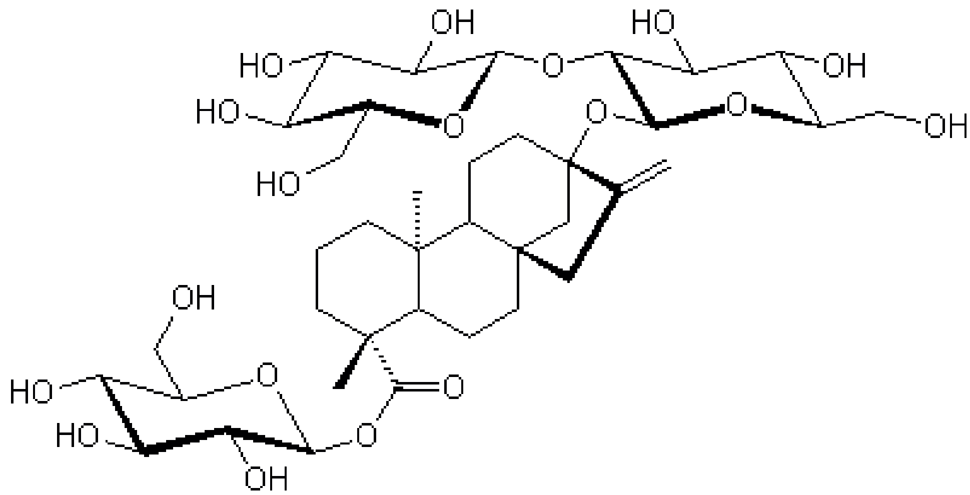

Figure 4. *The molecule of stevioside (De Muylder et al, 1998)*

which is obtained by different extraction methods of sweet stevioside and rebaudioside A from green powder (Abou-Arab et al, 2010). Still, on the market, but also in the food and baking industry the mostly applied of the above mentioned products is stevia white powder (Wallin, 2007; Amzad Hossain et al, 2010, Goyal et al, 2010) ie. the powder of extracted and concentrated mixtures of two most abundant stevia glycosides, stevioside and rebaudioside A (Makapugay et al, 1984). In order to obtain a white stevia powder, the classical method of extraction of stevioside by process of maceration and heat extraction is applied. Mentioned classical methods of extraction show numerous disadvantages of which the most important being a longer time period, relatively low efficiency of the extraction process, higher energy consumption, increased solvent usage and more released heat as well (Vinatoru, 2001). Also, Abou-Arab et al (2010) in the study on the efficiency of various conventional methods of extraction of steviol glycosides from the stevia leaves namely: a) by using water, b) methanol and c) methanol/water (4:1), determined that use of water in the extraction achieve efficiency up to 98% of removed stevioside and so produce "natural product" (Nishiyama, 1991). Methanol appears to be used in extraction process, presumably to improve extraction efficiency and facilitate the separation of individual steviosides (Brandle, 1998). But primarily, because of the extraction process productivity increasing necessity, in recent years begins intensive application of unconventional extraction methods such as the method of high intensity ultrasound, which finds its greatest advantage in increasing the yield and quality of the extracted compounds (Wang and Weller, 2006). Also, the fact of the economic justification of the high intensity ultrasound application (relatively low-cost method), its simple utilization and significant efficiency must not be neglected (Alupului et al, 2009). Alupului et al (2009) determined the benefits of applying high intensity ultrasound as a method for the extraction of stevioside from the stevia dry leaves compared to conventional thermal extraction techniques. Specifically, the application of high intensity ultrasound of the amplitude 50, 80 and 100% and input power of 750W increased rate of stevioside content in the samples on the maximum value in the time period of less than five minutes. The increase of the ultrasonic fields power in the above mentioned amplitudes did not show any visible effects of the concentration of stevioside (additional increase of concentration or decrease of the stevioside extraction time period), but in the application of higher power ultrasound as the main limiting disadvantage should be considered the risk of degradation of the extracted compound (Alupului et al, 2009).

The impact of high intensity ultrasound on the antioxidant compounds content

Phenolic compounds in food and food products have gained great popularity by the discovery of their significant antioxidant activity and a number of potential beneficial effects that may have on human health from cancer disease prevention to occurrence and prevention of cardiovascular diseases (Pearson et al, 1999; Auclair et al, 2008). In general, fruits and vegetables are the most important sources of different types of beneficial phenolic compounds (Markowski and Plocharski, 2006), and fruit species that we can point out as an extremely rich source of phenolic compounds is an apple (Boyer and Liu, 2004). As already mentioned, part of apple fruit which is the richest in phenolic compounds is a thin layer just below the skin of apple fruit (Ceymann et al, 2012). Therefore, from the processing aspect in the production of different apple products (apple juice, apple filling , jams, etc.) it is very important to include also the apple skin. During the production of for example the apple juice a significant part which remains after the milling and pressing the apple fruit (apple pomace) is a very rich source of phenolic compounds and can serve as a good source for the extraction of phenolic compounds (Četković et al, 2008), which can then find their wide application in food, various pharmaceutical (drugs) and cosmetic production (Peschel et al, 2006; Sandhu and Joshi, 1997; Gullon et al, 2007). Conventional methods of polyphenolic compounds extraction from fruits are based on the maceration process that shows many disadvantages especially in industrial production, and also the process itself is very expensive mainly because it requires expensive equipment (Virot et al, 2010). Precisely from the above mentioned aspect, using of high intensity ultrasound in the extraction of phenolic compounds with increased efficiency is increasingly being used, but still largely just at the level of laboratory experiments (Chemat et al, 2008; Cuoco et al, 2009). The main advantage of such extraction is much reduced period of extraction process. The conventional methods can take up to several days while applying high intensity ultrasound complete extraction can be done in a few minutes (Cuoco et al, 2009). In the study conducted by Virot et al (2010) the conventional method of phenolic compounds extraction from apple pomace and extraction of phenolic compounds using high intensity ultrasound are compared.

The results of this study indicate significant efficiency difference of the compared extraction methods (conventional and high intensity ultrasound). Specifically, it was determined that high intensity ultrasound can significantly improve the rate of released analytes from plant cells in comparison with the conventional extraction method. The yield of polyphenolic compounds extracted by ultrasound was even 20% higher in just 45 minutes of application.

Table 1. *Composition (mg/100g of apple pomace) and comparison of final extracts (Virot et al, 2010)*

	Conventional extraction	Ultrasound-assisted extraction
Flavan-3-ols	223.70	301.14
Procyanidins	190.13	266.09
DPn (procyanidins)	4.4	4.6
Dihydrochalcones	174.16	182.55
Phenolic acids	48.46	49.37
Flavonols	99.70	105.35
Extraction time	45 min	45 min
TPC*	769	964

DPn: average degree of polymerization
* mg catechin equivalent per 100 g of apple pomace

CONCLUSION

The application of minimum food processing methods such as the high intensity ultrasound finding widespread use in various technological processes of food processing, from the replacement for conventional thermal treatments, sterilization and pasteurization to the extraction of specific plant compounds (phenols, glycosides etc). The process of pasteurization could not be a fully replaced by applying the high intensity ultrasound because it is proved to be effective only for some of the microbial population. For the complete decontamination and for the removal of high resistant microorganisms it is recommended to combine the application of high intensity ultrasound with heat and/or pressure: thermosonification, manosonification and manothermosonification. High intensity ultrasound has proven to be very effective as a extraction method of different compounds in comparison with conventional extraction methods based on the maceration process and heat treatment. The basic advantage of high intensity ultrasonic extraction is significantly shorter time period required for the extraction of different compounds among others stevioside and phenolic compounds, as well as a higher rate and yield of the extraction process. It is important to emphasize that the application of high intensity ultrasound does not indicate any degrading or reducing rates depending on the content of extracted compounds, in this case, phenolic compounds and stevioside. Described method of minimal foods processing provides a great advantage mainly in order to preserve the quality of foods in terms of preserving important antioxidant components.

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