

## STABILIZATION OF SEA BUCKTHORN (*Elaeagnus rhamnoides*) TURBID JUICES

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### Abstract

*This paper reviews the technological solutions used to stabilize the sea buckthorn (*Elaeagnus rhamnoides*, syn. *Hippophae rhamnoides*) juice. Sea buckthorn is a nutraceutical crop of growing interest due to the health benefits of its berries active ingredients - essential (unsaturated) fatty acids, vitamins, antioxidants, and minerals. The turbid juice produced from the whole berries without enzymatic clearance has a higher biological value due to the extraction of the main active ingredients. However, the turbid juice made from sea buckthorn berries has poor physical and chemical stability. The lipids from berry pulp, rich in monounsaturated palmitoleic acid (16: 1n-7), separate from juices and are prone to rapid oxidation. Syneresis also occurs due to the low water-holding ability of the pulp polysaccharides. Oxidation reduces the content of the antioxidant (pro) vitamins. Stabilization is done by physical homogenization and/or utilization of additives with emulsification and/or antioxidant characteristics. The paper analyzes and discusses the advantages and drawbacks of the existing solutions.*

**Key words:** sea buckthorn, turbid juice, syneresis, oxidation, stabilization, homogenization, additives.

### INTRODUCTION

Sea buckthorn, *Elaeagnus* (synonym *Hippophae*) *rhamnoides* (L.) A. Nelson is a thorny deciduous shrub recently domesticated (Kalia et al., 2011). Long-period sea buckthorn berries were harvested from nature (Yang et al., 2009). Domestication and establishment of orchards with this robust shrub tree were driven by its ecologic (Constandache et al., 2016) and economic importance (Gatlan & Gutt, 2021). The ecologic importance of sea buckthorn results from its ability to colonize and stabilize marginal lands, including those from cold and dry areas or reclaimed spoil mines (Baig et al., 2021; Li & Schroeder, 1996; Zhao et al., 2013). The ability of sea buckthorn to grow well in poor soil is directly related to this robust shrub being both an actinorhizal plant and an endomycorrhizal plant (Zhou et al., 2017). As an actinorhizal plant, sea buckthorn has the ability to form symbiotic N<sub>2</sub>-fixing nodules with actinomycetes from *Frankia* genera (Diagne et al., 2013). The formation of symbiosis with mycorrhizae fungi supports phosphorus

acquisition from immobile soil sources and the development of N<sub>2</sub>-fixing nodules and other beneficial rhizobacteria (Yang et al., 2018).

The economic importance of the sea buckthorn results from the high content of its fruits and leaves in bioactive ingredients - (pro)vitamins, essential (poly)unsaturated fatty acids, and antioxidant phytonutrients (Ren et al., 2020).

Due to its high content in healthy related compounds, sea buckthorn has been utilized for millennia as a nutraceutical/medicinal plant in Europe, China, and India (Suryakumar & Gupta, 2011; Wani et al., 2016).

Sea buckthorn leaves are occasionally used to prepare healthy infusions. The sea buckthorn fruits (berries) are the main edible parts used for nutraceutical purposes. Because of their acidic taste and short shelf life, sea buckthorn berries are not consumed in a fresh state (Alexandrakis et al., 2014). Usually the berries are converted into turbid juice prepared from the flashy pulp/pericarp and in a seed oil preparation, obtained by the mechanical extraction of the edible oil from the seeds (Beveridge et al., 1999). The pomace is further used as a source

for tocopherols and carotenoids, extracted with supercritical CO<sub>2</sub> (Kitrytė et al., 2017; Mihalcea et al., 2021), for the production of soluble dietary fiber (Hussain et al., 2021) or for recovery of natural lipophilic antioxidants (Patra et al., 2022).

Turbid juice needs stabilization because of its heterogeneous composition, including suspended microparticles and hydrophobic and hydrophilic soluble fractions. The turbid juice separated during storage in three phases, with the oily one on the upper surface and sedimented particles on the bottom (Beveridge et al., 1999). This work reviews the technological solutions used to stabilize the sea buckthorn turbid juice. Initially, we detail the technical problems associated with the composition of the turbid juice and its peculiar rheology. Further, we describe the physicochemical processes used for turbid juice stabilization. We address the innovative solutions at the end of the paper, including those described in the published patents.

### Active ingredients in the sea buckthorn turbid juice

The diverse agro-pedo-climatic conditions promote the accumulation of different bioactive compounds in sea buckthorn berries and leaves. Romanian cultivars, differentiated in a separate subspecies, *E. (H.) rhamnoides* subsp. *carpatica* accumulated in the berries five carotenoids,  $\beta$ -carotene, cis- $\beta$ -carotene,  $\beta$ -cryptoxanthin, lutein, and zeaxanthin. The antioxidant activity of extract from these Romanian cultivars correlated well ( $r = 0.96$ ) with the total flavonoids. The antibacterial activity against *Bacillus cereus* and *Pseudomonas aeruginosa* was higher in the sea buckthorn leaves (Criste et al., 2020).

The pulp oils from sea buckthorn cultivated in the Indian Himalayas had a high content of unsaturated fatty acid (65-75%), palmitoleic acid (16:1n-7), being the most abundant (32-53%) (Ranjith et al., 2006). The cultivars from *E. (H.) rhamnoides* subsp. *mongolica* that has been grown in Poland (51.59° N, 20.139° E) accumulated more monounsaturated fatty (palmitoleic, 16:1n-7; cis-vaccenic, C18:1 n-7; oleic C18:1 n-9) compared to polyunsaturated fatty acids (linoleic C18:2 n-6) (Teleszko et al., 2015). The berries from *E. (H.) rhamnoides* cultivated in Turkey accumulated a higher

proportion of palmitoleic acid (47.3%) in the pulp (Cakir, 2004).

Despite variations due to subspecies - cultivars and the agro-pedoclimatic conditions, the presence in the pulp of a large amount of palmitoleic acid, in combination with different types of antioxidants, hydrophobic (carotenoids, flavonoids), and hydrophilic (hydroxycinnamic acids, ascorbic acid) is reported in all published studies - Table 1.

Table 1. Composition of the sea buckthorn fruit pulp

Active ingredients	Content (mg.g <sup>-1</sup> fresh weight)	Reference
Palmitoleic acid (16:1n-7)	2.54-15.92	(Yang & Kallio, 2001)
	4.27-16.7	(H. Kallio et al., 2002)
	2.64	(Cakir, 2004)
	4.95-23.76	(Dulf, 2012)
Total carotenoids	0.01 -0.18	(Andersson et al., 2009)
	0.02 -0.40	(Bal et al., 2011)
	0.17	(Pop et al., 2015)
Tocopherols	0.03-0.21	(Jiménez-Escrig & Sánchez-Muniz, 2000)
	0.06-0.14	(Heikki Kallio et al., 2002)
Flavonoids	3.54-10	(Bal et al., 2011)
	1.68-8.59	(Heinaaho & Julkunen-Tiitto, 2011)
Ascorbic acid	1.4-30	(Ranjith et al., 2006)
	0.25-25	(Bal et al., 2011)
Hydroxycinnamic acid	0.151-0.402	(Hajazimi et al., 2016)
Caffeic acid	0.03.6-0.087	
Ferulic acid	0.015-0.15	
p-Coumaric acid	0.10-0.265	

From 65 to 85% of the sea buckthorn berry flesh pulp is converted into turbid juice by (mechanical) squeezing the fruits. The turbid juice proved to have significant health benefits due to its high content in lipophilic and hydrophilic antioxidants and  $\omega$ -7 palmitoleic acid (Ciesarová et al., 2020). For decades, sea buckthorn has been recognized for its effect on cardiovascular diseases and associated metabolic disorders (Olas, 2016). The immunomodulatory and anti-inflammatory effects were confirmed by several studies (Ren et al., 2020). Also, *in vitro* studies on transformed cells and *in vivo* studies on laboratory animals confirmed the anticancer activity of sea buckthorn (Olas et al., 2018).

Palmitoleic acid reduces the risk of type II diabetes by increasing insulin sensitivity (Hu et al., 2019). Serum palmitoleate acts as a lipokinin (Merino et al., 2016) and prevents harmful effects of metabolic syndrome (Talbot et al., 2014; Tricò et al., 2020). Sea buckthorn pulp oil enhanced the immunity of immunosuppressed

mice due to a significant prebiotic effect on probiotic bacteria producing post-biotics, i.e., short-chain fatty acids (Zhang et al., 2021).

The carotenoids present in high quantity in turbid sea buckthorn juice synergize the palmitoleic effect on type II diabetes and metabolic syndrome. Sea buckthorn is an accessible source of carotenoids, especially zeaxanthin (Tudor et al., 2020). Carotenoids levels in serum were inversely correlated with type II diabetes (Marcelino et al., 2020) and metabolic syndrome (Beydoun et al., 2018; Matsumoto et al., 2021). This inverse correlation is higher for zeaxanthin (Sugiura et al., 2015). The hydrophilic antioxidants present in the sea buckthorn turbid juice are also active against type II diabetes (J.-S. Zheng et al., 2020) and metabolic syndrome (Liu et al., 2019). The anticancer activity of the sea buckthorn extracts was directly correlated with the level of ursolic acids (Grey et al., 2010), flavonol glycosides (Enkhtaivan et al., 2017) and flavonoid aglycones, including quercetin, isorhamnetin and kaempferol (Guo et al., 2017).

Recently, the low polarity fraction from sea buckthorn juice was reported to have low cytotoxicity for the normal cell and induce DNA damage apoptosis in cancer cells (Marciniak et al., 2021). This low polarity fraction from sea buckthorn juice contains triterpenoids, acylated triterpenoids, phospholipids and their derivatives, and saponins (Marciniak et al., 2021).

The lipophilic and hydrophilic antioxidants from sea buckthorn were reported to affect amyloid-beta (A $\beta$ ) levels directly. Therefore sea buckthorn could be efficient in Alzheimer's disease (Dong et al., 2020). The neuroprotective effect of sea buckthorn was also demonstrated against oxidative stress damage on neural tissue (Shivapriya et al., 2015), including the oxidative stress resulting from reperfusion following ischemia (Godeanu et al., 2020) and for iron-induced epilepsy (Ladol & Sharma, 2021).

### Instability factors of the sea buckthorn turbid juice

This unique combination of active ingredients with high biological value also represents the source of an intrinsic instability - Figure 1.

The sea buckthorn turbid juice has a low thermal stability. This thermal instability is related to the instability of several components with high

biological value, such as flavonoids, terpenoids, and carotenoids, and the dehydration reaction of soluble sugars in the presence of high-level ascorbic acids (Li et al., 2014). Polyphenols/hydroxycinnamic acids from the sea buckthorn juice tend to be more thermostable than carotenoids, flavonoids, and antioxidants (Ursache et al., 2017).

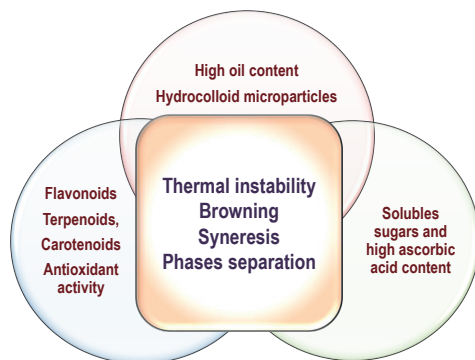


Figure 1. The instability factors of sea buckthorn turbid juice related to its specific composition

The formation of the 5-hydroxymethylfurfural (5-HMF) is associated with a non-enzymatic browning and a decrease in the level of ascorbic acid (Xu et al., 2015). HMF formation by dehydration of the soluble carbohydrates starts at a temperature lower than 60°C (Constantin et al., 2019). In sea buckthorn juice, HMF does not contribute to forming the Maillard type aroma/taste compounds and is considered a contaminant (Constantin et al., 2019; Rannou et al., 2016). The maximum allowed level of HMF in juice is 5 mg.L<sup>-1</sup> (Damasceno et al., 2008).

The formation of HMF could be associated with forming a more harmful contaminant, acrylamide (Constantin et al., 2019). However, the temperature promoting acrylamide formation is higher than 130°C (Constantin et al., 2019). The high oil content of the turbid juice, combined with the low water-holding ability of hydrocolloid microparticles, determine syneresis and phase separation (Beveridge et al., 1999).

### Sea buckthorn turbid juice stabilization treatment

Several different mechanical treatments were proposed to stabilize the sea buckthorn turbid juice. The majority of these are inspired by the stabilization of the milk, a food system that is

similar in terms of the presence of hydrophilic and lipophilic phases. Such treatments target the formation of a more stable suspension by generating smaller lipid droplets and reducing the particle dimension. However, sea buckthorn turbid juice is a much more complex system. It is not a simple emulsion; it is a suspoemulsion that includes both hydrophobic fractions and suspended particles. Each of the proposed treatments has some drawbacks.

Homogenization at 100 MPa in a piston homogenizer Panda PLUS 2000 (GEA Niro Soavi S.p.A., Parma, Italy) stabilizes the emulsion. Still, it generates a lighter and yellowish color that influences customers' acceptance (Aaby et al., 2020).

Better stabilization was obtained by using dynamic high-pressure microfluidization (DHPM) by using a Microfluidizer® (model M-110EH, Microfluidics Inc., Newton, MA, USA) (Abliz et al., 2021). The difference between a piston homogenizer and a microfluidization equipment is related to the dimensions of the space wherein the high shearing force occurs. The space is limited to a homogenizing valve that separates the high-pressure chamber from

the atmospheric pressure in a piston homogenizer. In a Microfluidizer® processor, the space is a whole interaction microfluidics chamber, with walls treated with high hardness materials (i.e., zirconia or diamond). Microfluidizer® is expensive equipment with low capacity that is not yet suitable for the food industry (Guo et al., 2020). Pretreatment is necessary to avoid clogging

Various emulsifiers were combined with a piston homogenizer to enhance the stabilization of the sea buckthorn turbid juice suspoemulsion. The most effective stabilization agents were sodium caseinate- and sugar esters. However, the best stabilization agents also influenced the antioxidant activity (H. X. Zheng et al., 2020). This decrease in the antioxidant activity is because the carotenoids from sea buckthorn juice bind to the proteins (Aprodu et al., 2017; Dumitraşcu et al., 2016).

Table 2 summarizes the homogenization treatments (including the combined homogenization and emulsifiers) intended to stabilize the turbid sea buckthorn juice. The effects and drawbacks are presented.

Table 2. Homogenization treatment proposed for the stabilization of turbid sea buckthorn juice

Treatment	Effect	Drawbacks	Reference
Homogenization in a piston homogenizer, 100 MPa	The reduction of the oily droplets and the polysaccharides particles increased the stability of the suspoemulsion	Lighter and yellowish color – consumer perception	(Aaby et al., 2020)
Microfluidization, up to 250 MPa, through a Z-shaped interaction chamber	Increased stability of the suspoemulsion, carotenoids released from droplets, higher antioxidant activity	Expensive equipment. technology is not yet ready for the food industry	(Abliz et al., 2021)
Emulsified agent + homogenization in a piston homogenizer	Increased stability of the suspoemulsions	Decreased the antioxidant activity due to conjugation	(H. X. Zheng et al., 2020)

Innovative approaches were proposed for the stabilization of the sea buckthorn turbid juice. The purified additives were replaced with different plant extracts – for example, the infusion from *Hibiscus* fruits (Heilscher, 1995). Other innovative approaches involved stabilization with extracts from sea buckthorn by-products - Figure 2.

Sea buckthorn berries juicing produce two types of by-products, leaves, and pomace. The fruits are very tightly bunched to the branches, and the

harvesting techniques generate significant amounts of leaves (Fu et al., 2014). The leaves have a higher antioxidant content than fruits (Olas, 2016).

The pomace is rich in pectin and antioxidants (Patra et al., 2022). Several patents claim utilization of the extracts from sea buckthorn by-products to stabilize the juice (Shi et al., 2019; Tian et al., 2018).

There are two main drawbacks of these solutions based on plant extracts. One is related to the

taste - the plant extract enhances the astringency and the bitterness of the turbid juice. The other one is related to the low reproducibility of the stabilization process. This low reproducibility

results from combining two highly variable products - the turbid juice and the plant extract (including the extract from the by-products of the sea buckthorn processing).

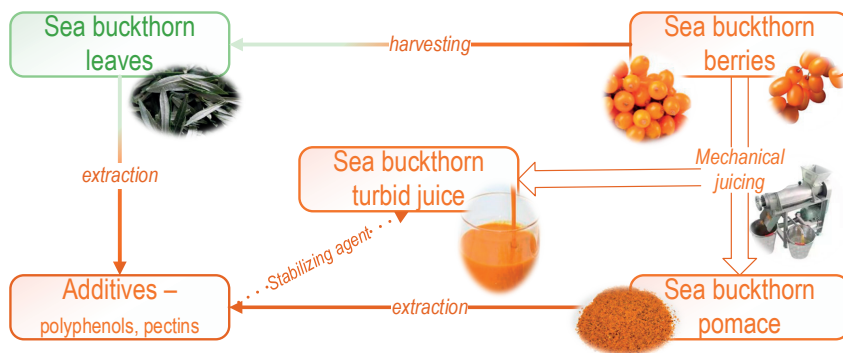


Figure 2. Illustration of the innovative approaches for stabilizing the sea buckthorn turbid juice by using extracts rich in polyphenols and/or pectin from sea buckthorn processing by-products

## CONCLUSIONS

The sea buckthorn turbid juice is unstable and prone to browning and phase separation. Stabilization of the sea buckthorn turbid juice is done by physical homogenization and/or utilization of emulsifiers.

Innovative solutions involve the use as additives for stabilization of the extracts from the by-products of the sea buckthorn processing.

Complexity of the turbid juice suspoemulsion determine low reproducibility of the stabilization process.

Because of the drawbacks of the existing solutions, new approaches are needed for sea buckthorn turbid juice stabilization.

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