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Development and Validation of *Aronia melanocarpa* Berry Recipes for Home Canning: Integrating Thermal Lethality Studies, Microbiological Safety, and Antioxidant Analysis

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DEVELOPMENT AND VALIDATION OF *ARONIA MELANOCARPA* RECIPES FOR HOME CANNING: INTEGRATING THERMAL LETHALITY STUDIES, MICROBIOLOGICAL SAFETY, AND ANTIOXIDANT ANALYSIS

by

Juan Diego Villegas Posada

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DEVELOPMENT AND VALIDATION OF *ARONIA MELANOCARPA* BERRY RECIPES FOR HOME CANNING: INTEGRATING THERMAL LETHALITY STUDIES, MICROBIOLOGICAL SAFETY, AND ANTIOXIDANT ANALYSIS

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University of Nebraska, 2024

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Aronia melanocarpa, also known as chokeberry, is gaining popularity for its high antioxidant content and health benefits, including reducing oxidative stress and inhibiting cancer gene expression. However, its characteristic bitterness and astringency limit its broader appeal. Chokeberry's low pH places it in the acid foods category, making it ideal for safe home canning. An acidic environment combined with heat treatment inhibits harmful bacteria like Clostridium botulinum. Home canning preserves food by sealing it in airtight containers and heating it to destroy spoilage microorganisms.

Developing safe and palatable recipes is essential for making chokeberries suitable for home canning. A statistical approach, surface response methodology, is used to optimize jam and salsa recipes through controlled sensory evaluations. Once recipes are finalized, thermal lethality studies determine their safety, with shelf-life studies assessing product stability over 12 to 18 months. Additionally, the project examines the impact of processing on the natural compounds in Aronia, particularly polyphenols. Antioxidant levels are measured using ABTS assays, as studies have shown that storage and processing can significantly reduce these beneficial compounds, though some, like proanthocyanidins, may increase after pasteurization (Etzel et al., 2015; Wilmore et al., 2015; Wilkes et al., 2014).

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Chapter 1. LITERATURE REVIEW

1. *Aronia Melanocarpa*

A. melanocarpa also known as chokeberries are part of the Rosaceae family; the plant is a deciduous shrub and can grow up to 3 meters (Ochmian et al., 2012). There are four species of chokeberries: *A. arbutifolia*, red chokeberry; *A. melanocarpa*, black chokeberry; *A. prunifolia*, purple chokeberry; and *A. mitschurinii*. However, there are different varieties of the species resulting from the domestication of the berries (Mahoney et al., 2019). The berries are ripe at the end of August and through September (King & Bolling, 2020). The color of the chokeberries varies from red to black, depending on the species; fruits with intense red and purple colors have a high concentration of anthocyanins, making these dark-colored fruits some of the most important sources of the compounds (Kahkonen et al., 2003).

1.1 Global production and market overview

Aronia berries, also known as chokeberries, have been gaining popularity due to their high antioxidant content and associated health benefits. The global market for *Aronia* berries is expected to experience steady growth, with a compound annual growth rate (CAGR) projected at approximately 3.3% from 2023 to 2030. The market value is expected to reach around USD 255.16 million by 2030. Poland is one of the largest producers of *Aronia* berries globally, contributing significantly to the market with a well-established cultivation system. The United States also has a strong production base, with the Midwest region being particularly active in growing *Aronia* berries. The U.S. market share in global exports is approximately 6.7%, with an export value of USD 199.4 million in 2023 (Tridge, 2024). Other notable producers include Canada, Chile, and several European countries like Serbia and Germany. The increasing demand for

functional foods and natural health products are driving the expansion of the *Aronia* berry market. The berries are utilized in various forms, including whole berries, juice, dried products, and extracts, catering to the food and beverage, pharmaceutical, and nutraceutical industries. Despite its growth, the *Aronia* berry market faces challenges such as limited consumer awareness and the bitter taste of the berries, which can restrict their appeal. However, efforts are being made to overcome these barriers through product innovation and targeted marketing strategies (Maximize Market Research, 2024).

1.2 Composition of *A. melanocarpa*

Chokeberries are composed of functional and structural components from which the bioavailability will depend on various factors: the place where it was grown, the form of consumption, the specific cultivar, the ripened state of the berry, and when it was harvested (Kulling & Rawel, 2008). Organic acids, proteins, and lipids give the fruit stability, while nutrients, polyphenols, fiber, and sorbitol (8.56g/100g) (Sidor & Gramza-Michałowska, 2019) account for the functional components that make *A. melanocarpa* desirable (King & Bolling.,2020). The carbohydrate content of the berries varies from 6.21 to 20.92g/100 g fresh weight basis (fwb) (Sidor & Gramza-Michałowska, 2019). It is divided into sugars, ranging from 16 to 18% (Kulling & Rawel, 2008), and fiber, ranging from 5.62g/100g (Kulling & Rawel, 2008) to 7.52g/100g (King & Bolling, 2020). While most of the sugars are extracted in the juice, the fibers are maintained in the pomace, and their concentration ranges from 57.8 to 71.5g/100 g dry weight basis (dwb) (Sidor & Gramza-Michałowska, 2019).

Berries are known for their low content of protein; in the case of the chokeberry, the protein contents of 3.7g/100g in the fresh fruit and 4g/100g to 24g/100g in the

pomace with arginine, tyrosine, histidine, lysine, cysteine, alanine, asparagine, serine, glutamic acid, and threonine being the principal amino acids of *A. melanocarpa* (Sidor & Gramza-Michałowska, 2019; King & Bolling, 2020). Fat is relatively low in the berries, with 0.09-0.17% fwb (Sidor & Gramza-Michałowska, 2019).

Vitamins and minerals present in chokeberries further promote the health benefits of the fruit and are presented in Table 1 (Jurendić & Ščetar, 2021); the mineral content of the fresh fruit is reported as 4-6 g/kg found in the form of ashes (Jurendić & Ščetar, 2021). Although toxic elements like lead, cadmium, mercury, and arsenic are found in the berries (Sidor & Gramza-Michałowska, 2019), the amount of these elements is low enough for the fruit to be safe for consumption (Cindrić et al., 2017). Vitamin C is the most abundant vitamin in *A. melanocarpa* (King & Bolling, 2020).

Organic acids present in the chokeberry are about 1-1.5% (Ochmian et al., 2012; Jurendić & Ščetar, 2021) of the fresh fruit are represented by the quinic, malic, shikimic, citric, oxalic, succinic, and isocitric acids (King & Bolling, 2020); some authors report malic acid as the most abundant, followed by citric and quinic (Kulling & Rawel, 2008), and other authors report quinic as the most abundant (293-591 mg/100g fwb) followed by malic (308-350mg/100g fwb) (King & Bolling, 2020). The pH resulting from the presence of the acids ranges from 3.3 to 3.9 (Kulling & Rawel, 2008); this will vary according to the cultivar used and the harvesting week of the fruit (Bolling et al., 2015). This inherent acidity makes *Aronia* berries well-suited for home canning applications, as it provides a natural defense against the growth of harmful microorganisms, namely *Clostridium botulinum* (Lewis, 1996).

Table 1. Vitamins and minerals present in *Aronia* berries

Vitamins and minerals	**Amount in 100 g**	**Percent Daily Value (%)**
Minerals (mg)		
Sodium (Na)	2.6	0.3
Calcium (Ca)	32	4
Potassium (K)	218	9
Magnesium (Mg)	16	7
Zinc (Zn	0.2	2
Iron (Fe)	0.9	8
Vitamins (mg)		
Thiamin (B1)	0.02	2
Riboflavin (B2)	0.02	2
Pyridoxin (B6)	0.03	3
Niacin	0.3	3
Pantothenic acid	0.3	6
Ascorbic Acid (C)	14	22
Tocopherols (E)	1.7	17
Folate / µg	20	10
Phylloquinone (K) µg	24	50

Note. Adapted from "“Jurendić, T., & Ščetar, M. (2021). *Aronia* melanocarpa Products and By-Products for Health and Nutrition: A Review. Antioxidants, 10(7), 1052”

Phenolic compounds are protagonists in biological activities, both in vivo and in vitro studies, and are considered the most important compounds present in the fruit, accounting for the health benefits that the berry presents. *A. melanocarpa* has a high concentration of procyanidins, anthocyanins, and phenolic acids (Kulling & Rawel, 2008). Kulling and Rawell (2008) stated that there are limitations when determining the amount of total phenolic compounds found in *Aronia* due to the wide range of analytical methods such as HPLC/DAD and HPLC/ESI-MS as well as the variations caused by external conditions mentioned previously. Various authors have reported values of the total phenolics; Kolesnikov and Gins (2001) reported 3440mg/100g dwb, Hudec et al.

(2006) found 3760mg/100g dwb, Kähkönen et al. (1999) determined the amount of total phenolic compounds as 4010mg/100g dwb and 4210mg/100g dwb in a posterior study, where anthocyanin was the most abundant polyphenolic compounds, mainly 3-galactoside, 3-glucoside, 3-arabinoside, and 3-xyloxide (Kähkönen et al., 2001). Nevertheless, various authors have found that polymeric proanthocyanins account for 66% of the fruit polyphenols, with (-) epicatechin (Oszmianski & Wojdylo, 2005) as the main compound of this group, followed by (+) catechin (King & Bolling, 2020).

1.3 Potential Health Benefits of *A. melanocarpa*

Aronia has shown significant potential for health benefits for different affections, including an increased antioxidant capacity when compared to 92 polyphenol food extracts (Kähkönen et al., 1999). This may be explained by its higher content of total polyphenols (Kähkönen et al., 2001). Wu et al. (2004) reported a total antioxidant capacity of 161 µmol of Trolox equivalents per gram of fresh fruit.

Studies have demonstrated that the polyphenols, flavonoids, and anthocyanins present in berries can modulate biomarkers of DNA damage; animal models show the action over various paths of the carcinogenic pathway, stabilizing the genome (Duthie, J., 2007). Anthocyanin-rich extracts of *Aronia melanocarpa* showed a 60% inhibition of colon cancer cell replication without affecting the growth and proliferation of healthy colonic cells (Malik et al., 2003). *Aronia* extracts showed the most potent inhibition compared to commercially prepared grape and bilberry anthocyanin-rich extracts (Zhao et al., 2004). Additionally, the aberrant chromatid cohesion, which causes chromosome instability and promotes cancer development, was decreased in tests with human blood-

derived lymphocytes in vitro; inhibition of superoxide radicals due to the intense free-radical scavenging action of the anthocyanins was observed (Gasiorowski et al., 1997).

Aronia melanocarpa natural juice reduced necrotic changes in rats' livers caused by acute exposure to carbon tetrachloride (CCL4) and inhibited the apparition of plasma aspartate transaminase and alanine transaminase caused by CCL_4 (Valcheva-Kuzmanova, 2004).

Kowalczyk (2003) reported a reduction of cadmium, also showing a reduction of the concentration of bilirubin and urea in blood serum, resulting in an overall reduction of cadmium accumulation in the animal’s liver and kidneys.

Anthocyanins present in chokeberry, specifically cyanidin 3-5diglucoside, show an inhibitory effect on dipeptidyl peptidase, which results in a reduction of body weight and blood glucose and a reduction of adipose tissues in diabetic mice (Yamane et al., 2016). Baum et al. (2015) found a reduction in body weight and obesity biomarkers in mice fed low-fat and high-fat diets compared to mice fed identical diets plus *Aronia Melanocarpa* juice concentrate supplementation. Some of the bioactive compounds responsible for these effects are (−)-epicatechin, chlorogenic acid, neochlorogenic acid, and cyanidin-3-galactoside (Banjari et al., 2017)

Aronia melanocarpa extracts have been tested in bovine endothelial cells and L-NAME-induced hypertensive rats with promising results inducing maximal nitric oxide production and nitric oxide synthase phosphorylation in the bovine endothelial cells which results in a decrease in risk of cardiovascular disease (Fromentin et al., 2016) and a 21% reduction of blood pressure, nitric oxide synthase upregulation, conjugated diene

reduction in the left ventricle and aorta and regulation of proinflammatory process in L-Name-induced hypertension in rats (Cevoba et al., 2017). In contrast, Bhaswant et al. (2016) studied rats with diet-induced metabolic syndrome by comparing the response to anthocyanins supplementation extracted from purple maize and chokeberry, resulting in a reduction in visceral adiposity index, total body fat mass, systolic blood pressure, improved glucose tolerance, liver, and cardiovascular structure and function.

The range of potential benefits of *Aronia* and its intense color has been creating a niche market worldwide, with European countries as the primary producers; the fruits are canned whole, the juice extract is used for jelly making or combined with other healthy fruits, and the natural colors are used in the food industry (Knudson, 2009). The high acidity makes chokeberries suitable for canning applications, eliminating the risk of contamination by the spore-forming bacteria *Clostridium botulinum.*

2 Home-Canning

Canning appeared in late 17th century France. Napoleon's government offered a generous reward to anyone able to create a sustainable method to preserve food due to the increased demand for nutritional food on the war fronts (Powell, 1917). The mission was to provide the French troops overseas with good food and respond to the needs of millions of starving families (Appert, 1810) shortly after the creation of the methodology, in 1810, Peter Durand patented the tin cans to reduce the costs of canning, and just two years after that, the first cannery was opened in the United States, increasing the demand for canned green peas (Bitting, 1909). Ezra Daggett brought the industrialization of

canning in America in 1819, whose expertise was on canning salmon, lobsters, and oysters in New York; however, the rapid growth of the industry was evident, preserves and table condiments were being canned in 1821 and in 1835 tomatoes started to being canned, followed by corn (1837). The industry's rapid growth led to studying the natural causes of food spoilage. According to Guy Lussac, a series of oxidation reactions dominated the spoilage mechanism, but from 1822 to 1895, Tyndall and Pasteur proved that there were microorganisms responsible for the spoilage of the foodstuff (Powell, 1917).

As technology advanced, different agents causing spoilage and diseases were found. Three types of foodstuffs were distinguished with each of the processing requirements for food safety: acid foods with a natural pH of 4.6 or below, acidified foods to which the addition of acid or acid foods reduced the pH to 4.6 or below, and low-acid foods that naturally have a pH greater than 4.6 (Anderson & Zhao, 2021).

Low-acid foods with a pH above 4.6 are particularly susceptible to the growth of *Clostridium botulinum*. This spore-forming bacterium produces botulinum toxin, the cause of botulism, a potentially fatal illness (Lewis, 1996). Thermal processing is essential to ensure the microbiological safety of low-acid canned foods. This involves heating the food to a specific temperature and time combination, known as the thermal process, to destroy all *C. botulinum* spores. The specific thermal process parameters for low-acid canned foods depend on the characteristics of the food, such as its density, viscosity, and acidity (Murano, 2014). Generally, low-acid foods require heating to at least 121°C (250°F) for at least 3 minutes to ensure botulinum spore destruction (Anderson & Zhao, 2021). Acidified foods, with a pH between 4.0 and 4.6, are less

susceptible to *C. botulinum* growth due to the acidic environment. However, other microorganisms, such as *Bacillus, Staphylococcus, Salmonella,* and *E. coli,* can still pose food safety risks (Erkmen, 2022). A combination of acidification and thermal processing is typically employed to ensure the safety of acidified foods. Acidification involves adding acids, such as vinegar or lemon juice, to the food to lower its pH. The desired pH level depends on the specific food and shelf life (Anderson & Zhao, 2021). Thermal processing, while not as crucial for acidified foods as for low-acid foods, still plays a role in destroying vegetative cells of spoilage microorganisms. Acid foods with a pH below 4.0 are inherently safe due to the highly acidic environment inhibiting most microorganisms' growth, including *C. botulinum*. However, good manufacturing practices (GMPs) are essential to prevent contamination during processing and handling (Barron & Fraser, 2013). *Aronia* berries, with a typical pH range of 3.3 (frozen berries) to 3.92 (juice), fall into the category of acid foods (King et al., 2022; Tolić et al., 2015).

The reason for canning is to ensure that the produce to be canned will maintain most of its nutritional value for a longer time. Most fruits and vegetables will have a reduction in the active compounds that support human nutrition, and some canned foods could be more nutritious than those in retail stores. However, to ensure that the food to be canned will be nutritious, steps and considerations must be taken to eliminate oxygen and enzymes and reduce the possibility of microbial growth, selecting adequate fruits, removing non-edible parts, using appropriate jars and lids, and processing the food for enough time and at the right temperature is critical. The temperature will vary according to the altitude where the fruit will be prepared, so the time used to treat the food will vary accordingly. Acidic food like *Aronia* hot-packing ensures the removal of oxygen from the

fruit and the jar. Leaving a sufficient head space in the jars where the food will be canned to allow the expansion of the fruits is necessary; in the case of jams, the USDA recommends one-fourth of an inch. The jars and the self-sealing lids must be sterilized in boiling water for 10 minutes. To reach a properly processed product, the water of the canner must be preheated to 180°F for hot-packed foods (USDA., 2009).

3 Surface Response Methodology in Food Science

The surface response methodology (SRM) is a combination of statistical and mathematical approaches for optimizing and understanding the functional relationship between a perturbation in a system and the response the system shows, allowing the scientist to reach the optimal condition of the process to be studied (Khuri & Mukhopadhyay, 2010). One of the advantages that make the SRM one of the most widely used statistical tools is the systematic approach for analyzing single or multiple variables on a response of interest and allowing the researchers to rapidly design experiments in the direction of the maximized or minimized response depending on the needs of the study, and generating a mathematical model that describes the behavior of the system (Bas & Boyaci, 2005). SRM can be designed for first and second-order systems. For first-order systems, the designs can be 2^k factorial, Plackett-Burman, and Simplex. On the other hand, the design for second-order systems can be 3^k factorial, Box-Behnken, and Central composite (CCD) types (Yolmeh, 2016).

The central composite design is a versatile experimental design approach used to understand the behavior of a system in the vicinity of the central point. It helps to

quantify the curvature and interaction of the effects of the input variables. This type of design is used in different sciences and engineering to understand processes and optimize their responses. The central composite design has three fundamental components: Factorial, axial, and central points. The factorial points are utilized to calculate both linear and quadratic effects of input variables, while the axial points are used to evaluate the curvature and interaction effects. The center points are included to estimate the pure error and to assess the repeatability of experimental measurements (Kidane, 2021). This combination of factorial, axial, and center points enable a thorough exploration of the response surface, providing graphical representations of the system's behavior that allow the researcher to approach the desired response. One strength of the CCD is using axial points to observe the system's behavior at the edges of the experimental limits. This provides information about how robust or sensitive the system is to the variations imposed on it. It first fits a first-degree model to get the initial information of the response. It allows the researcher to see if the variables are essential to the response. The additional points (Axial) facilitate determining the optimal operating conditions, elevating the model to a second-degree one (Khuri & Mukhopadhyay, 2010).

The versatility of the Surface Response Methodology (SRM) and the Central Composite Design (CCD) becomes evident in the food industry, where these powerful statistical tools are employed to optimize and enhance food processing, product development, and quality control. The methodology has been used for the optimization of extraction, drying, microencapsulation, and controlled release, enzymatic hydrolysis and clarification, blanching processes, production of microbial enzymes and other metabolites (Yolmeh, 2016), baked products development, cooking and roasting parameters,

extrusion, fermentation, and formulation of new recipes and component composition of foods (Kidane, 2021).

4 Sensory Evaluation of Food Products

Sensory evaluation represents one of the most crucial steps in food product development; it became one of the pillars of food production around the 1950s when "golden tongue" experts evaluated certain products they had long experience in tasting. Wine, brew masters, flavorists, and other experts helped improve product quality assurance and development. For two decades, most of the sensory evaluation techniques could not be analyzed using statistical methods, which posed a problem in reproducing results and determining the validity of the data obtained. In 1974, a method called the quantitative descriptive analysis was developed and was the first to be evaluated by analysis of variance (ANOVA) (Gacula, 1997). Since then, more than 20 new methods derived from this have been developed by scientists. Most companies have in-house methodologies developed by their experts (Kemp et al., 2018).

Sensory evaluation consists of evoking, measuring, analyzing, and interpreting the responses to products perceived through the senses of sight, smell, touch, taste, and hearing (Kemp et al., 2018). This type of evaluation comprises a set of techniques that allow the researchers to make precise measurements of the human responses to food, conducting the test in a manner that minimizes the biases that the participants may have due to branding or other information that may affect how a person perceives the tested food (Lawless & Heymann, 1999).

For sensory evaluation, different considerations are made to eliminate bias. For example: 1) the panelists usually are divided into independent test booths to eliminate the influence of other panelists in their results; 2) the samples use randomized numeration so the labeling of the samples prevents panelists from forming ideas based on the label, but only what they taste and perceive only; and 3) the order of the samples may vary from panelist to panelist to asses for the changes in perception that the panelist may have due to the order of the samples and the components of the food. It is a common and recommended practice to standardize the samples to the same temperature, volume, and spacing in time to avoid changes in the perception due to differences in the sample. If a sample is hotter or colder or has more volume than the other, the perception may be affected by the influence of these factors on the flavor or mouthfeels, making the data untrustworthy (Lawless & Heymann, 1999).

Although sensory evaluation is based on human perception, it is still a quantitative discipline in which gathering information is crucial to establish meaningful connections between product characteristics and human sensory perception. For this, evaluators allow the panelists to give numerical food ratings according to the panelist's preferences. Next, the analysis must be done from the data obtained from the panelists; most of the time, this data has a wide variability, which comes from different factors like mood, sensibility, experience with the product, and even panelists' motivation. Even screening measurements will not entirely nullify the variation, and the evaluator must use statistical methods to analyze the evaluation data, which is where the experiment design has influence.

5 Antioxidant Analysis of Foods

Antioxidants are chemical compounds in foods and are defined as “Those substances present in a relatively low amount in foods, which inhibit, delay or control oxidation of food components, consequently preventing spoilage and extending the shelf life of foods.” (Zeb, 2021). Antioxidants can trap free radicals, which are atoms or groups of atoms that cause the oxidation of compounds in food that cause the aging and deterioration of foods. Some of the most common oxidants are oxygen, ozone, hydrogen peroxide, nitrous acid, and nitric or nitrous oxides. Free radicals can be formed from lipids, proteins, carbohydrates, organic and inorganic compounds, and metal atoms and occur from internal cellular nutrient transformation processes. The antioxidants can be enzymatic or non-enzymatic. Vitamins, carotenoids, phenolic compounds, minerals, and sulfur-containing organic compounds are well-known non-enzymatic antioxidants of which the phenolic compounds have been gaining much popularity due to their traditional and modern medicinal uses (Kolesnikov & Gins, 2001)

To determine the antioxidant activity, spectrophotometric, potentiometric, and electrochemical methods have been developed: oxygen radical scavenging capacity assay, total radical-trapping antioxidant parameter (TRAP), Trolox equivalent antioxidant capacity (TEAC), ferric reducing antioxidant power (FRAP), total antioxidant activity (TAA) and ABTS/PP (2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)) assay are just few examples (Zeb, 2021).

The ABTS/PP assay uses potassium persulfate (PP) to initiate the production of $ABTS^{\bullet+}$ radical cation. Then, the food to be analyzed is combined with the cation, which causes the cation (deep bluish-green) to be decolorized by antioxidants (Nenadis, 2004).

One advantage of the ABTS/PP assay is the stability of the PP and ABTS oxidation reaction in a pH range of 2.0 to 15.0 (Ilyasov et al., 2020).

6 Shelf-life Studies

Shelf life is the length of time that a food can remain in a wholesome, consumable state, retains the desired sensory, chemical, physical, functional, or microbiological characteristics, and comply with any label declarations when stored and distributed under the recommended conditions (Taormina & Hardin, 2021; Man, 2015). Shelf-life studies are conducted to determine the factors that will promote the deterioration of a product over time. It also helps to determine the optimal storage conditions to ensure that the product will maintain its quality and safety. These studies help manufacturers determine the most appropriate packaging and transportation methods, accounting for the temperature and humidity changes that the product may be subject to (Taormina & Hardin, 2021). Every country has regulations for the shelf-life of products to ensure that consumers will have wholesome and safe food. Food producers must inform consumers of the expiration dates and the adequate storage conditions to ensure that they will not get sick; plus, they avoid recalls that could result in a loss of consumer trust and popularity (Man, 2015).

Shelf-life testing is done by collecting samples of the food, freshly produced and packed as the consumer would buy it, and then storing it in the conditions it will experience on the shelves. Periodically, the scientist or producer will study changes in microbial profile, rancidity, color, smell, and all the critical characteristics that the product has. Shelf life is determined according to the number of days the food has kept all the acceptable conditions and characteristics established by the company and

governmental regulations (Taormina & Hardin, 2021). However, the process of shelf-life testing is expensive and time-consuming; accelerated shelf-life testing (ASLT) can be a valuable tool to reduce the time spent on testing and allow producers to develop new products that meet the safety standards efficiently. For this, the temperature at which the food is stored is raised, assuming any deterioration will be apparent sooner and the shelf-life will be shortened. Using extrapolation, the shelf-life at the recommended conditions can be calculated. Although practical and precise, ASLT has limitations, the most important being that they are product-specific. Additionally, the temperature can cause physical changes; relative humidity variations can cause unexpected results, and freezing causes a concentration of components (Man, 2015).

7 Thermal Lethality Studies

To create safe recipes for home canning, the canning process must be evaluated to determine if food is being submitted to enough thermal lethality to avoid the presence of spoilage and pathogens, using reliable microbial thermal-death-time information to calculate the decimal reduction of microorganisms (Etzel, 2015), the decimal reduction D_T is the time in minutes at a constant temperature to reduce 90% of the initial microbial population in a sample (Stoforos, 2010). As the temperature changes, D_T changes as well; the Z-value is referred to as the thermal resistance constant, and the temperature increase is needed to modify the D_T by a factor of ten. To calculate these values, the temperature of an inoculated food sample is monitored through the canning process, and the data obtained from microbial loads and temperatures can be used to calculate the D_T

and Z-values. For instance, the Z-value for E. coli O157:H7 is 5.56°C at 82.2°C with a DT = 0.00008 min (Etzel, 2015).

The process lethality, F, is the number of minutes at a particular temperature that will allow a specific reduction of the microbial load in the food. The microorganism and the temperature of the process influence the process's lethality. The values of Z and D_T are used together in the Ball formula to find the F (Equation 1).

$$\frac{FF^{zz}}{TTTTTTTT} = \int_{dd_{aa}}^{dd_{bb}} 10^{\frac{TT(tt)-TT_{rrrrrr}}{zz}} dddd \quad (1)$$

The Ball formula method is a classical method used in the thermal processing industry to calculate the lethality of thermal processes for canned foods. It assumes that the rate of destruction of microorganisms (D_T) in a food product is proportional to the product temperature (T) (TechniCAL, 2019). The Ball formula method can calculate the F of a thermal process for a given product and design thermal processes for a new product. The Ball formula method is a relatively simple method for calculating the lethality of thermal processes. However, it is essential to note that the method does not consider factors such as the size and shape of the product container, the agitation of the product during processing, and the presence of other ingredients in the product. These factors can affect the actual lethality of a thermal process. The formula method assumes that the Z-value of the microorganisms is constant over the temperature range of the process (Stoforos, 2010).

8. SUMMARY

Home canning is a valuable procedure to ensure the long-term nutritional value of foods. The acid (pH below 4.0) food process has specific thermal processing parameters to prevent spoilage and contamination. *Aronia* berries, with a pH range of 3.3 to 3.92, are ideal for canning, providing a natural defense against harmful microorganisms. *Aronia* is rich in compounds that promote health benefits. To develop safe *Aronia* recipes for home canning, it is crucial to ensure that the canning process provides adequate thermal lethality to eliminate spoilage organisms and pathogens. The first step to developing the recipes is to identify from a set of recipes with variations in the ingredients which one will be preferred among the consumers; tools like the surface response methodology combined with sensory analysis can provide the researchers insights into what characteristics are desirable. Additionally, it is essential to understand if the potential health benefits provided by the chokeberry due to its antioxidants are maintained after thermal treatments. Finally, microbial stability must be tested to ensure a wholesome and safe product for the public that will perform home canning.

9. OBJECTIVES

Although there is widespread interest in using *Aronia* berries in more homemade products due to their health benefits, few scientifically tested recipes exist. This thesis aims to develop home canning recipes and processes for chokeberries that are easy enough to make but delicious and safe. Therefore, the overarching objectives for this thesis are:

- Objective 1: To adapt and validate an existing jam recipe from the National Center for Home Food Preservation with *Aronia* berry as the primary ingredient, ensuring its microbiological safety and quality through appropriate thermal treatment. Additionally, to assess the acceptability of the product by sensory evaluation and its shelf life based on physicochemical characteristics.
- Objective 2: To adapt and validate an existing salsa recipe from the National Center for Home Food Preservation with *Aronia* berry as the primary ingredient, ensuring its microbiological safety and quality through appropriate thermal treatment. Additionally, to assess the acceptability of the product by sensory evaluation and its shelf life based on physicochemical characteristics.

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Chapter 2. JAM RECIPE DEVELOPMENT AND VALIDATION WITH *ARONIA* BERRY AS THE PRIMARY INGREDIENT, ENSURING ITS MICROBIOLOGICAL SAFETY AND QUALITY THROUGH APPROPRIATE THERMAL TREATMENT

ABSTRACT

This study focused on developing and validating a jam recipe using *Aronia* berries (*Aronia melanocarpa*), known for their high antioxidant content. The goal is to ensure the jam's microbiological safety and quality through proper thermal treatment while preserving its nutritional value and sensory appeal. The process begins with adapting a USDA-approved jam recipe by replacing traditional fruits with *Aronia* berries. Critical parameters such as the degree of Brix (sugar content) and pH are measured and adjusted to ensure safety. Sensory evaluation using a surface response methodology (SRM) helps fine-tune the recipe. Various formulations were assessed by a sensory panel for texture, flavor, sweetness, aftertaste, and overall acceptability, aiming to balance taste and safety that cater to consumer preferences.

Thermal lethality studies are conducted thoroughly to determine the optimal heat treatment for ensuring microbiological safety. The jam is heated to achieve a 5-log reduction in pathogens such as *Escherichia coli*. Thermal death time (D-value) and z-value are utilized to establish effective thermal processing parameters. Microbial analysis is carried out to validate the jam's safety by testing for spoilage microorganisms, ensuring it meets microbiological standards. The antioxidant capacity of the *Aronia* berry jam is also analyzed for antioxidant activity using assays, such as the ABTS radical scavenging assay, to determine the jam's retention of nutritional benefits during processing. Shelf stability was assessed through accelerated storage studies to monitor sensory attributes, microbial load, and antioxidant capacity over time. This aids in establishing the jam's shelf life and providing proper storage guidelines.

This study develops and validates a jam recipe using *Aronia* berries, known for their high antioxidant content. The goal was to ensure microbiological safety through proper thermal treatment while preserving nutritional value and sensory appeal. Using a USDA-approved recipe as a base, this study measured critical parameters such as Brix and pH and conducted sensory evaluations to fine-tune the recipe. Thermal processing studies achieved a lethality equivalent greater than 5-log reductions in pathogens such as *Escherichia coli*, ensuring safety. The jam's antioxidant capacity and shelf stability were also validated to establish home canning and storage guidelines.

1. INTRODUCTION

The growing consumer interest in functional foods and natural health products to prevent future health problems has driven the exploration and utilization of fruits rich in bioactive compounds (Canning, 2010). *Aronia* berries (*Aronia melanocarpa*), commonly known as chokeberries, have gained attention for their remarkable health benefits, primarily attributed to their high levels of antioxidants, polyphenols, and anthocyanins (Sidor & Gramza-Michałowska, 2019). These small, dark berries possess potent antioxidant properties, reducing oxidative stress and potentially lowering the risk of chronic diseases such as cardiovascular diseases, cancer, and antidiabetic potential (Ren et al., 2022).

Despite their nutritional advantages, *Aronia* berries have remained underutilized in mainstream food products, primarily due to their astringent taste and limited consumer familiarity (Kang et al., 2018). However, there is an interest by the *Aronia* industry to promote the use of this berry in value-added products to increase demand. This study addresses this gap by developing a jam recipe incorporating *Aronia* berries as the primary ingredient. Jam, a well-known fruit preserve, offers an ideal medium to introduce *Aronia* berries into the diet in a palatable form while retaining their nutritional benefits; this is caused by the amount of soluble dry matter that must be present in the jam, which influences the perception of characteristics of the food (Fügel et al., 2005).

The safety of home-canned products is crucial for consumers; the improper preservation of foods can lead to harmful microorganisms, including pathogens such as *Escherichia coli* (Dufort et al., 2014). Although chokeberries have a low pH (3.3 – 3.9) (King et al., 2022), which eliminates the risk of contamination by *Clostridium Botulinum*

(USDA, 2010), appropriate thermal treatment is required to ensure the microbiological safety and quality of the canned product.

Furthermore, the jam's sensory qualities, such as flavor, texture, and overall acceptability, are crucial for consumer acceptance. Sensory evaluation using a surface response methodology (SRM) helps refine the recipe to balance safety and taste (Yusof et al., 2021). Additionally, this chapter explores the antioxidant capacity of the *Aronia* berry jam after thermal processing, providing insights into how its potential health benefits are retained.

This chapter will cover the comprehensive process and robust analysis of adapting the jam recipe, conducting thermal lethality studies, performing microbial and antioxidant analyses, and assessing shelf stability. The findings will contribute to the knowledge of the safe and effective use of *Aronia* berries in home-canned products, highlighting their potential as a functional food ingredient and promoting its health benefits to encourage the use and inclusion of berries in households' diets.

2. MATERIALS AND METHODS

2.1 *Aronia* Jam Preparation

A recipe for blackberry jam with added pectin was selected from the National Center for Home Food Preservation, and the blackberries were substituted with *Aronia* berries. According to the manufacturer's instructions, canning jars (16 fl oz, 473 ml, Ball Canning Corporation, Muncie, IN) were sterilized, and the two-piece canning lids were put into boiling water for ten minutes before filling. Fully ripe berries were sorted and washed to remove stems or caps. The berries were blended at high speed for ten minutes

in a professional blender (Ninja model BL610, SharkNinja, Inc., Needham, MA) to ensure the homogeneity of the berry paste. The berry paste was measured in a saucepan, and pectin and butter were added. The mixture was heated to a rolling boil until the surface was covered by bubbles, constantly stirring. Sugar was added, and the mixture was stirred. The mixture was allowed to reach a rolling boil and was kept boiling for one minute, then it was removed from the heat, and the foam present at the top was removed with a metal spoon. The jam was immediately placed in the sterile jars, leaving ¼ inch of headspace. The rims of the jars were wiped with a clean, damped paper towel, and the two-piece metal canning lids were closed slightly tight. The jars were placed in a 23-liter boiling water canner (BWC) (Victorio VKP1130; Victorio Kitchen Products, Orem, UT) and processed for 15 minutes. The cooked jars were removed from the BWC and let cool undisturbed overnight. The seals were checked to ensure all the jars were airtight.

2.2 Brix Degrees Measurement

After cooking each batch, samples were taken to measure the jam's solids content. A digital refractometer (Hanna, HI96801., Hanna Instruments, Smithfield, RI) was used, and it was calibrated with distilled water to ensure a zero reading. The refractometer's prism was cleaned and dried, and then a small amount of jam was placed on the prism to cover it completely. After that, the equipment was turned on, and the measurements were taken.

2.3 pH Measurement

After cooking each batch, samples were taken to measure the pH of the jam to ensure it had the appropriate acidity to be suitable for canning in a boiling water canner. The samples were diluted with deionized water in a 1:1 proportion to ensure no change in the pH of the sample. The samples were transferred to plastic cups, and the probe from the digital pH meter (Orion Star, A111., Thermo Fisher Scientific, Waltham, MA) was placed in the jam. The reading was allowed to stabilize and then recorded.

2.4 Experimental Design

Nine recipes were obtained (Table 2) using a Box-Wilson Circumscribed Central Composite Desing, using an alpha of 1.414 according to the two factors affecting the response of the analysis: *Aronia* and sugar for the jam, as shown in Table 1.

Table 1. Formulation of *Aronia* Jam

Factor	Parameter	Unit	Low Limit	High Limit
A	Amount of *Aronia*	Grams	1800	2188
B	Amount of Sugar	Grams	1610	2240

Table 2. Combination of *Aronia* Jam formulation with coded variable levels for experimental design

Recipe	Amount of *Aronia*, g (X_1)	Amount of Sugar, g (X_2)
1	1800 (-1)	1610 (-1)
2	2188 (1)	1610 (-1)
3	1800 (-1)	2240 (1)
4	2188 (1)	2240 (1)
5	1994 (0)	1925 (0)
6	1994 (0)	1925 (0)
7	1719 (-1.414)	1925 (0)
8	2268 (1.414)	1925 (0)
9	1994 (0)	1480 (-1.414)
10	1994 (0)	2370 (1.414)
11	1994 (0)	1925 (0)
12	1994 (0)	1925 (0)

2.5 Sensory Evaluation and Recipe Optimization

Sixty untrained panelists were recruited for the *Aronia* jam sensory testing. Nine random numbers were assigned to each recipe to avoid biases from the panelists. The panelists entered a booth where they received nine recipes, one at a time. The panelists had to evaluate the products of 5 different parameters on a 9-point hedonic scale: texture, flavor, sweetness, aftertaste, and overall likeness. The results from the sensory were evaluated and fitted to a quadratic model with interactions to estimate the best combination of sugar and *Aronia.* The models were evaluated using ANOVA to ensure their goodness of fit. The IRB project # for this part of the research was 23535.

2.6 Thermal Lethality Studies

Once the sensory evaluation data was evaluated an optimized recipe was created with the model equations and this recipe was used for thermal lethality studies. Two-

piece lids were modified by perforating a hole in the center of the lid, and stuffing boxes (Pouch receptacle C5.2; TechniCAL, New Orleans, LA) were fitted to the lids. The thermocouples (Temperature distribution/Free lead wires; TechniCAL, New Orleans, LA) were inserted into the stuffing boxes in a manner that the measuring tip of the thermocouple was in the geometrical center of the jars (16 fl oz, 473 mL; PT), around 7 centimeters from the top. The jam was cooked following the procedures explained in section 2.1 of this document, replacing the two-piece lids with modified ones. Once the jars were filled and the modified lids adjusted, the thermocouples were connected to a data logger (CALPlex Datalogger; TechniCAL, New Orleans, LA) equipped with Calsoft 6 software (TechniCAL, New Orleans, LA). The data jars were placed in a boiling water canner with preheated water at 82°C. When the water reached boiling point, the cooking time started, and the product was held for fifteen minutes according to USDA recommendations. The temperatures were recorded by the software every minute, and the heating and cooling lethality of the process was calculated using the software, which implemented the ball formula method. The total lethality of the process was calculated by solving the integral of the ball formula with the trapezoidal method.

2.7 Antioxidant Analysis

Three samples were taken at different steps while preparing the jams to understand the antioxidant compounds' behavior. The first sample was taken after blending the *Aronia* to obtain a baseline of the antioxidant capacity of fresh fruit. The second sample was taken right before the jam was poured into the jars for canning. The third sample was taken after the canning process was completed, and the jam was cooled

to room temperature. The reagents were prepared 12 hours before the assay. Their preparation consisted of dissolving 0.181 g ABTS (Sigma Aldrich, A1888-2G, stored at 4 °C) in deionized water, making the volume 50mL to obtain a 7mM ABTS solution. Once the solution was ready, the ABTS cation radical-containing solution (ABTS•+) was prepared by dissolving 33.1 mg potassium persulfate (Sigma Aldrich, 216224-100G) in the above 7mM ABTS solution; the mixture was vortexed, ensuring the complete dissolution of the potassium persulfate. For the assay, the ABTS•+ was diluted with ethanol to achieve an absorbance of 0.70 (±0.01) cm-1 at 734 nm. Ethanol was used as a blank. The samples taken at different times were dissolved in ethanol 10mg/mL concentration. 200 µL of the 10 mg/mL sample was mixed with ethanol into 1.5 mL of the ABTS•+ solution (control). The mixture was incubated in the dark at room temperature (20-25°C) for 6 minutes, and the absorbance was read at 734 nm for the jam dissolved in ethanol and the control. Finally, the scavenging activity was calculated using the formula:

$$SSSSSSSSSSSSSSSSSS\ SSSSaaSSSSSSaaaa\ (\%) = \frac{AAAAAAAATTAAAAAAAATT\ AATT\ AAAAAAdTTAAcc - AAAAAAAATTAAAAAAAATT\ AATT\ AAAAssssccTT}{AAAAAAAATTAAAAAAAATT\ AATT\ AAAAAAdTTAAcc} \times 100$$

(1)

2.8 Shelf-life Studies

For the shelf-life study, the jars from each run of the optimized recipe of jam were stored at room temperature (20-25°C) for one month. Once the month passed, the canned jam were evaluated for aerobic plate count with dilutions 10^{-1} to 10^{-3} using yeast and mold (YM) Petrifilm and aerobic plate counts (APC) Petrifilm (3M; Saint Paul, MN).

3. RESULTS AND DISCUSSION

3.1 Model Fitting and Analysis of Variance

The primary goal of this project was to develop safe and appealing recipes for *Aronia* jam. A circumscribed central composite design was used to determine nine recipes that would help describe a surface response. Table 3 shows the mean response values for sensory analysis of chokeberry jams.

Table 3. Mean sensory analysis data for the sensory evaluation of *Aronia* berry jam

	Factors		Responses				
Recipe	*Aronia* content (g)	Sugar content (g)	Texture	Flavor	Sweetness	Aftertaste	Overall liking
1	1800	1610	5.35	5.15	4.78	4.8	4.92
2	2188	1610	5.42	6.05	4.68	5.47	5.56
3	1800	2240	5.12	5.36	4.68	4.78	5.12
4	2188	2240	4.81	5.07	5.14	4.73	4.71
5	1994	1925	4.42	5.29	5.05	4.85	4.83
6	1994	1925	4.62	5.31	5.12	4.72	4.91
7	1719	1925	5.31	5.54	4.81	4.8	5.07
8	2268	1925	5.36	5.71	4.53	5.15	5.51
9	1994	1480	5.44	5.8	4.32	5.19	5.41
10	1994	2370	5.35	5.25	4.33	4.58	5
11	1994	1925	4.51	5.4	5.1	4.88	4.84
12	1994	1925	4.82	5.25	5.05	4.79	4.8

The data from Table 3 was analyzed to determine which recipe among the tested ones was the most preferred. Recipe number 2 had superior flavor and overall liking to the others, with values of 6.05 for flavor and 5.56 for overall liking.

Table 4. Adjusted R-squared values for Tested Models Across Parameters

Parameter	Linear	Quadratic	Logarithmic	Square Root	Inverse	Box-Cox	Quadratic with Interaction	GAM
Texture	-0.13	0.73	-0.14	N/A	N/A	N/A	0.77	N/A
Flavor	0.29	0.25	0.28	N/A	N/A	N/A	0.95	N/A
Sweetness	-0.2	-0.016	-0.2	0.08	0.12	0.06	0.92	0.047
Aftertaste	0.64	0.66	0.65	N/A	N/A	N/A	0.91	N/A
Overall	0.12	0.36	0.10	N/A	N/A	N/A	0.97	N/A

Fitting each parameter response to a model equation was necessary to understand the behavior of the system. Additionally, the quality of the fit of the model was measured using ANOVA. Table 4 provides a summary of the adjusted R^2 values. R^2 is a statistical measurement that represents how well the model describes the variability of the response data around its mean (Piepho, 2019). The quadratic models with interaction terms provided the best fit for all the parameters. Having the model fit, it is possible to obtain the model equations for each of the parameters:

Texture:

$$\text{Log (Texture)} = 1.6114 - 0.0203 \cdot X_1 + 0.1301 \cdot X_1^2 - 0.0825 \cdot X_2 + 0.1447 \cdot X_2^2 - 0.1499 \cdot X_1 \cdot X_2 - 0.2552 \cdot X_1^2 \cdot X_2^2 \quad (2)$$

Flavor:

$$\text{Log (Flavor)} = 1.6874 + 0.0598 \cdot X_1 + 0.0182 \cdot X_1^2 - 0.0976 \cdot X_2 - 0.0064 \cdot X_2^2 - 0.4333 \cdot X_1 \cdot X_2 + 0.0079 \cdot X_1^2 \cdot X_2^2 \quad (3)$$

Sweetness:

$$\text{Log (Sweetness)} = 1.5641+0.0139\cdot X_1+0.0502\cdot X_1^2+0.035\cdot X_2-0.0485\cdot X_2^2+0.2293\cdot X_1\cdot X_2+0.1266\cdot X_1^2\cdot X_2^2 \quad (4)$$

Aftertaste:

$$\text{Log (Aftertaste)} = 1.5873+0.08\cdot X_1+0.0473\cdot X_1^2-0.1121\cdot X_2+0.0219\cdot X_2^2-0.2825\cdot X_1\cdot X2+0.0502\cdot X_1^2\cdot X_2^2 \quad (5)$$

Overall liking:

$$\text{Log (Overall)} = 1.6161+0.0461\cdot X_1+0.0487\cdot X_1^2-0.0816\cdot X2+0.0357\cdot X_2^2-0.4112\cdot X_1\cdot X2-0.0573\cdot X12\cdot X_2^2 \quad (6)$$

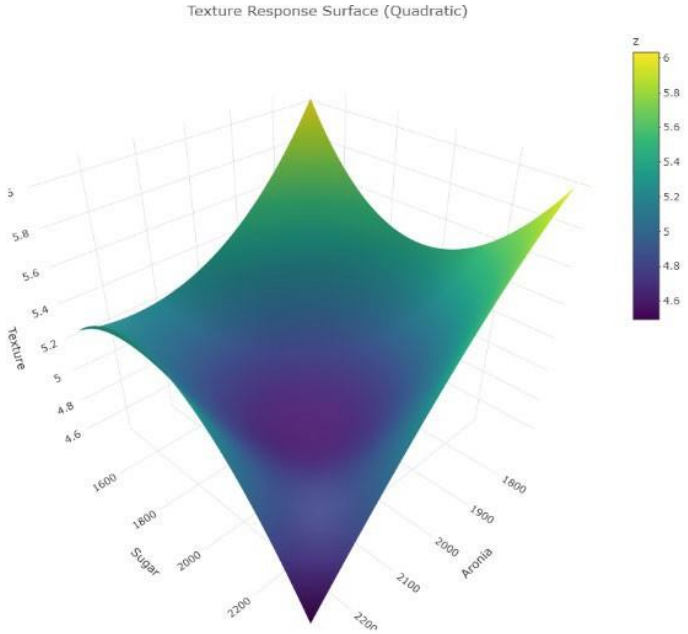

***Figure 1**. Interaction between variables for texture*

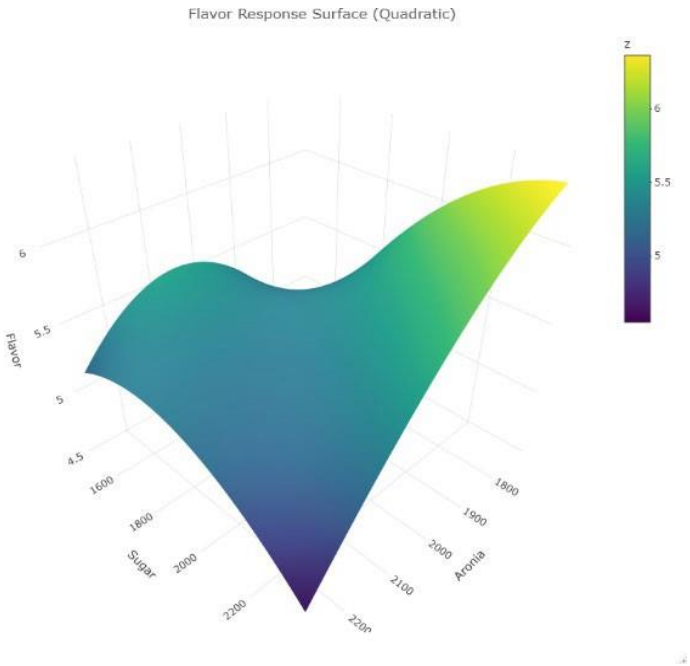

***Figure 2**. Interaction between variables for flavor*

These equations describe the relationship between the parameter studied and the factors X_1 (*Aronia*) and X_2 (Sugar), including their interactions and quadratic terms. With this equation, it is easy to evaluate the optimized conditions for the best responses of the target parameter (Merkle & Rosseel, 2018).

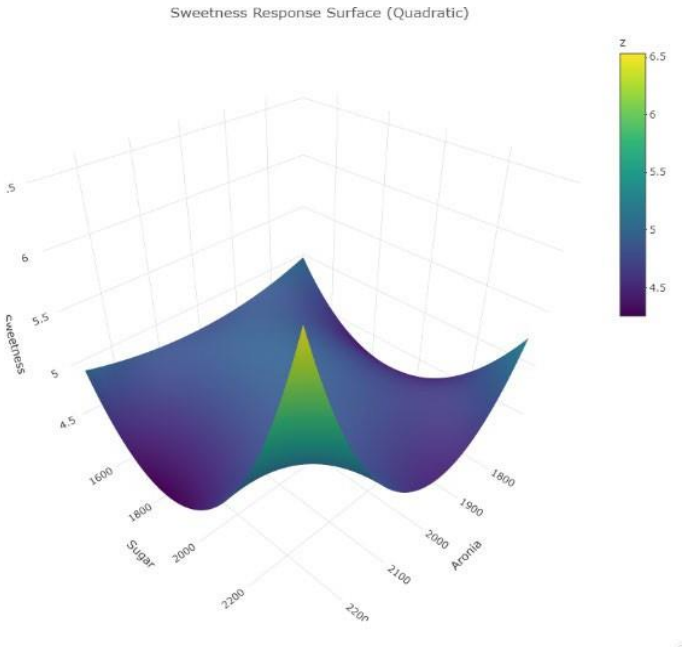

***Figure 3.** Interaction between variables for sweetness*

The logarithmic transformation helps stabilize the variance and makes the residuals more customarily distributed (Benoit, 2011). A prediction of the optimal quantities of *Aronia* and Sugar was obtained using the model equations 3-7.

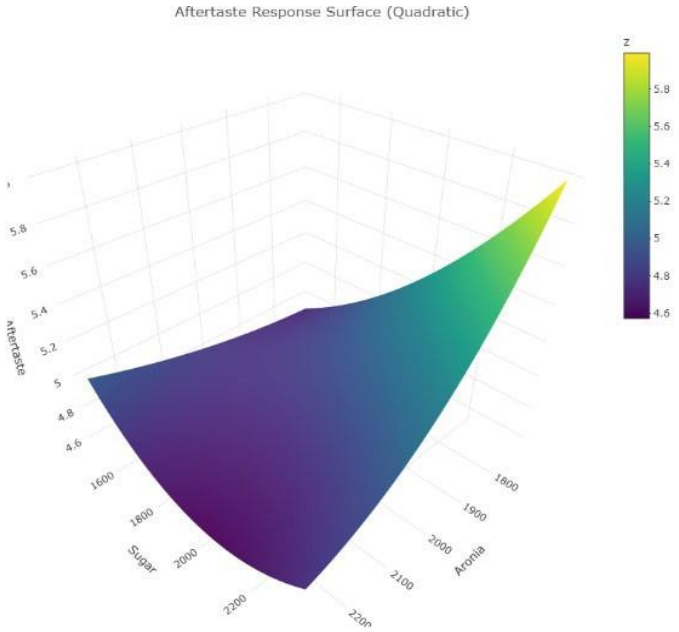

Figure 4. *Interaction between variables for aftertaste*

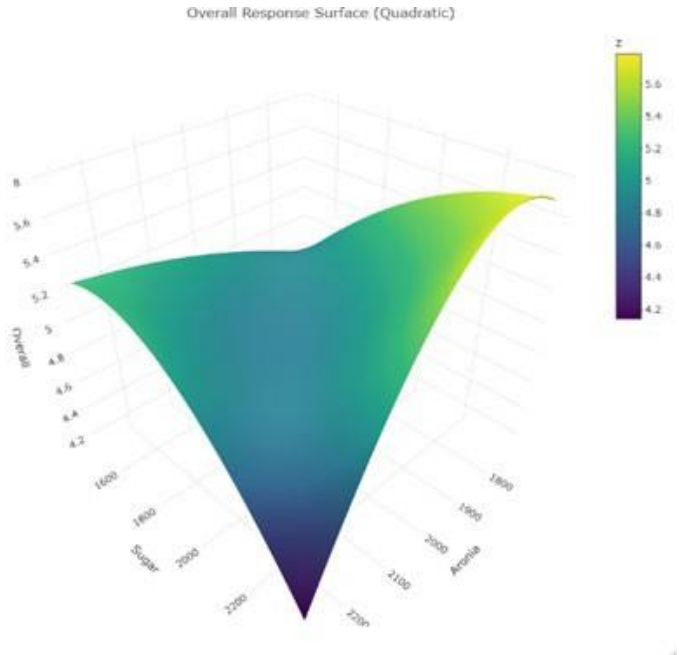

Figure 5. *Interaction between variables for overall liking*

Figures 1 to 5 present the interactions between the study variables and their influence on the parameters' responses. The graphs use the blue color to indicate low ratings of the parameter, whereas yellow represents high ratings. Figure 1 shows that low quantities of sugar have approximately the same effect on the texture as high quantities of sugar when the chokeberry is in low quantities. The center of the graph shows a deep section, indicating that the panelists did not enjoy the texture as much; chokeberries are known for their tartness and astringency (Kang et al., 2018), which may be mitigated by the sugar when the *Aronia* is added in small amounts. Figure 2 shows how the *Aronia* content influences the flavor of the jam. When the amount of *Aronia* increases, the graph curves down with the lowest point at the highest *Aronia* content. Surprisingly, the samples' sweetness peaks when the two variables are added in the highest quantities, and additionally, it improves when *Aronia* is added in the maximum quantity and sugar in the minimum when both are at the minimum and when *Aronia* is the minimum. Sugar is at the maximum quantity (Figure 3). The influence of the chokeberry on the aftertaste is evident. Figure 4 shows that the most preferred point is when 1800 grams of *Aronia* are present in the mixture, and the acceptance steadily diminishes when the berry amount increases. From Table 3, the panelists graded the best jam with high contents of *Aronia* (2188) and low content of sugar (1610 g), followed by jams with medium sugar content (1925 g) and the highest *Aronia* (2268 g). However, Figure 5 indicates that combinations of low *Aronia* content with high sugar content have the overall highest scores, followed by low sugar content and high *Aronia* content.

The jam samples received mixed feedback from the panelists. Many appreciated the sweetness levels and noted that the thicker consistency in some samples was

favorable. However, a common criticism across samples was the gritty texture, which was off-putting for many. This texture issue often detracted from the overall enjoyment, with some panelists mentioning that the seeds and skin pieces contributed to an unpleasant mouthfeel. The aftertaste was another point of concern; while some samples had a balanced aftertaste, others were described as astringent, bitter, or even sour, leaving an undesirable lingering effect. A few panelists also noted a dry or "earthy" aftertaste, which was not appealing. Despite these issues, some samples were praised for their balance of sweetness and tartness, though the overall texture and consistency were still areas where improvements were suggested.

Table 5 Summary of Analysis

Parameter	Mean	Standard Deviation	Minimum	Maximum	Correlation with Initial Temperature (r)
Rate of Heating (f_h)	41.46 min	7.13 min	32 min	58 min	0.37
Rate of Cooling (f_{cl})	72.34 min	8.12 min	62 min	92 min	
Lethality during Cooling (F_{cl})	2015.89 min	719.93 min	807.41 min	3164.23 min	
Lethality during Heating (F_h)	3788.06 min	1952.28 min	913.31 min	8349.85 min	0.41
Total Lethality (F_{Total})	5803.95 min	2491.44 min	1776.3 min	11058.57 min	0.38
Initial Temperature ($T_{Initial}$)	95.83°C	1.83°C	91.59°C	98.82°C	

3.2 Thermal Lethality

The optimal estimated value for *A. melanocarpa* obtained with the model equations was 2268 grams for *Aronia*, and for sugar, it was 1650.8 grams; these values are very close to the most preferred recipe from the sensory evaluation results, repetition #2, which had 2188 grams of *Aronia* and 1610 grams of sugar. Table 5 presents the summary of the data analyzed for this system.

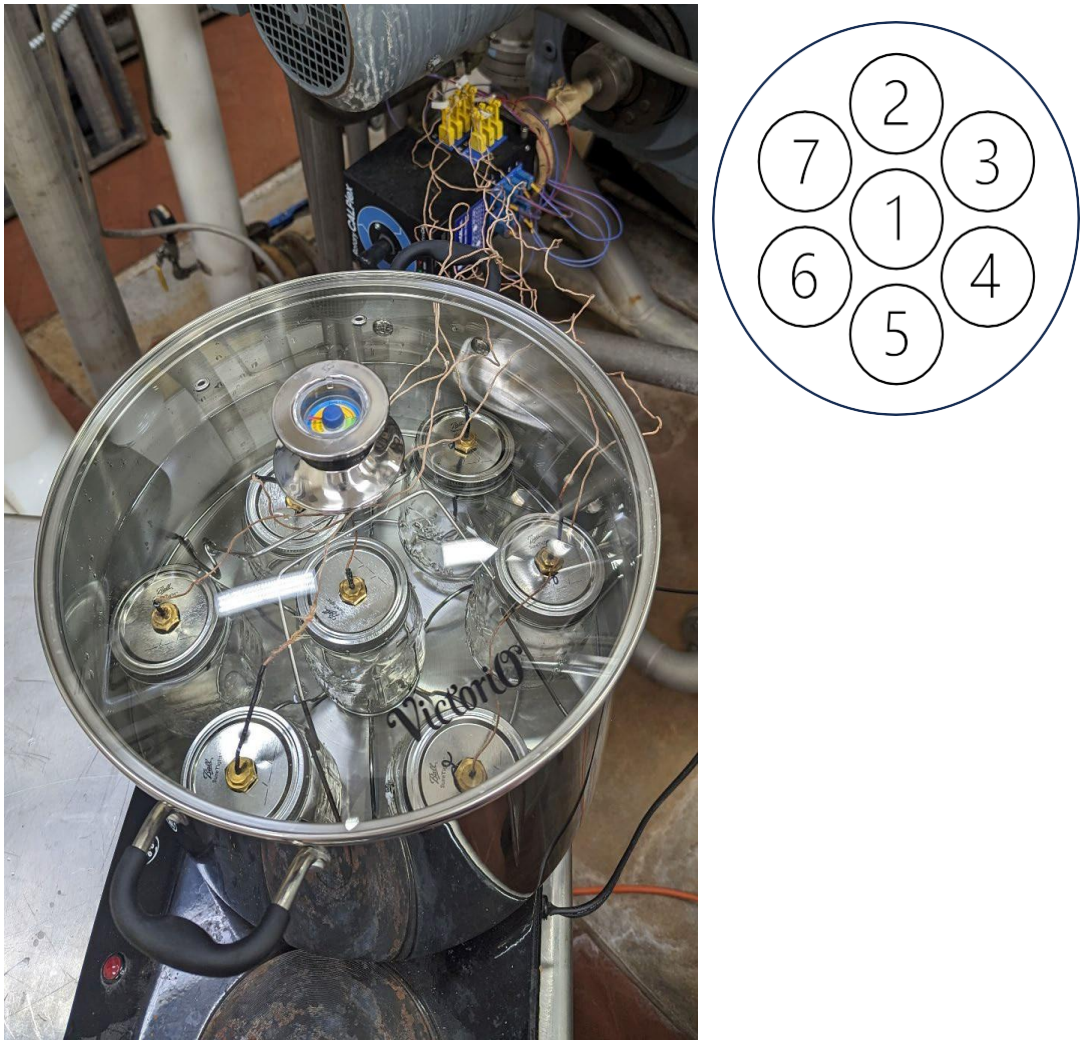

Figure 6. *Layout of Thermocouples*

Thermal lethality studies were carried out based on the estimated values for the optimized recipe. The study aimed to determine the total lethality (F $_{82.2°C}$) of the thermal process selected for the jam. Additionally, the data was analyzed for each sample to understand how the product's initial temperature and the heating medium at the start of the thermal processing influence the total lethality of the process (Table 6).

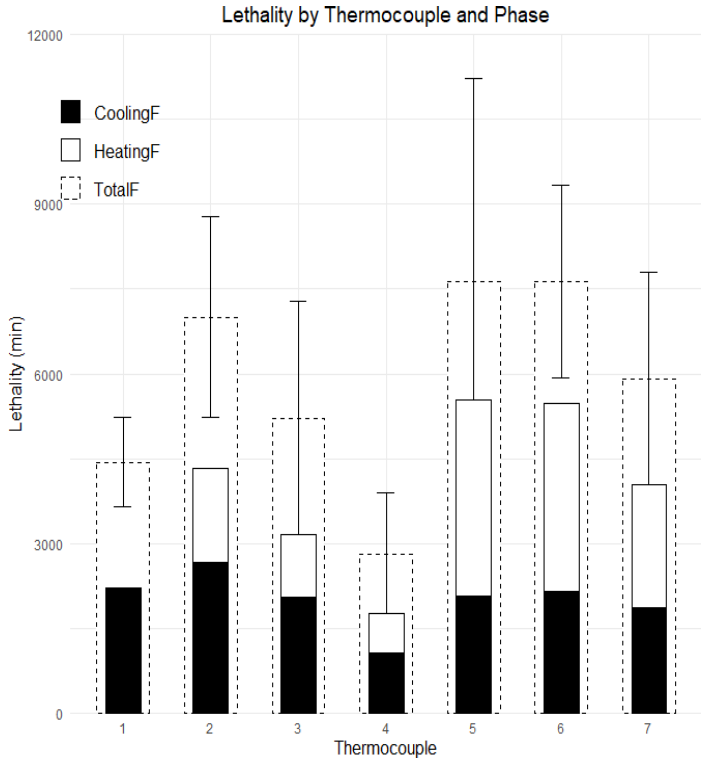

Figure 7. *Thermal Lethality by Thermocouple location and process step*

The jars were positioned as Figure 6 shows, so the lethality could be calculated for each position, allowing an easier statistical analysis. Figure 7 presents the mean F $_{82.2°C}$ (in minutes) observed at various thermocouple locations during different phases of

the thermal process: Cooling $F_{Cl\text{-}82.2°C}$ (black bars), Heating $F_{H\text{-}82.2°C}$ (white bars), and Total $F_{82.2°C}$ (striped bars). The graph illustrates the variability and distribution of lethality measurements across seven thermocouple locations. Error bars represent the standard deviation, indicating the variability within each phase at each thermocouple location. It can be observed that the cooling phase of the thermal process has a lower lethality overall (Figure 7), with a minimum of 1986.09 minutes, and a maximum of

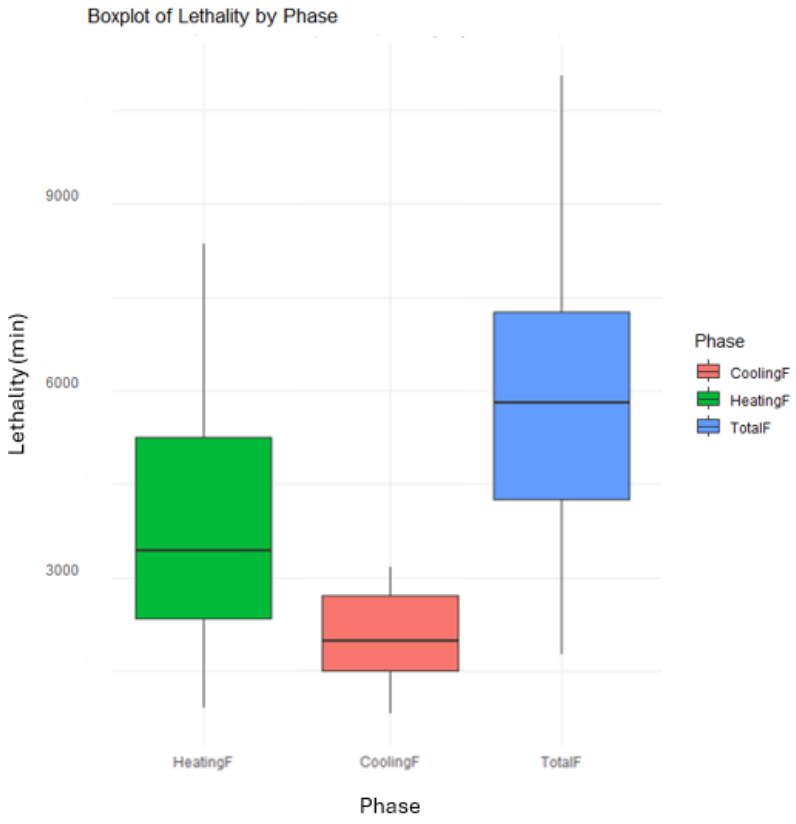

Figure 8. *Comparison of Lethality across cooling, heating and totality of the process.*

3164.23 minutes, and additionally, it showed a more compact distribution with fewer outliers (Figure 8).

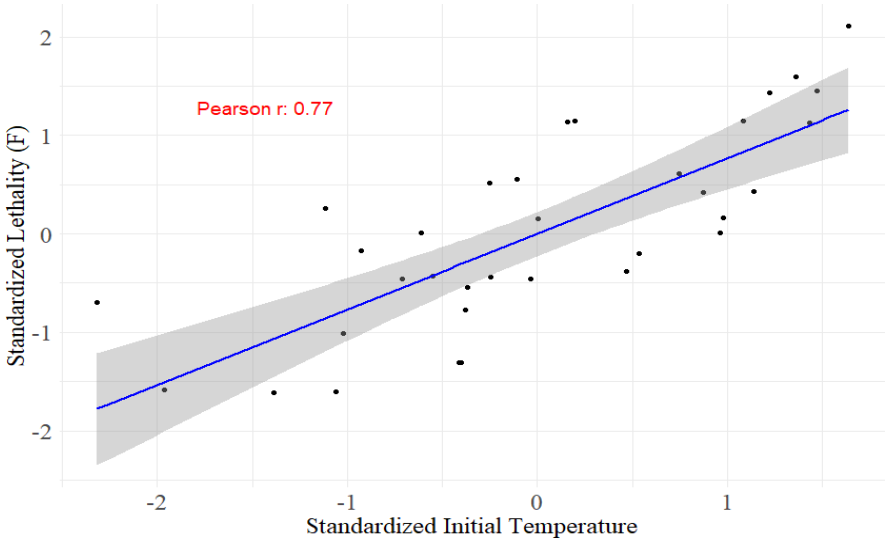

Figure 9. *Correlation between initial temperature and total process lethality*

The results indicate that the cooling phase contributes less to the overall lethality than the heating phase. In the case of the heating phase, the minimum is 913.31 minutes, and a maximum of 8350.85 minutes, with more variability than cooling, with a mean of 3500 min (Figure 8). The total lethality of the process had a minimum of 1776.27 minutes and a maximum of 11058.59 minutes; the distribution has the largest variability among the three phases of the thermal processing, however, in all cases, all the samples

reached a reduction of microorganisms larger than the 5-log reduction required (Etzel et al., 2015).

Table 6 shows the relationships between the total lethality and the other variables. Figure 9 shows that the Initial Temperature of the samples shows a strong correlation with the total process lethality (0.768); as the initial temperature increases, the total lethality will also tend to increase. The canner temperature influences the total lethality directly but has a lesser influence than one of the initial temperatures of the samples (0.46). From this information is clear that a combination of the initial temperature of the sample and the initial temperature of the heating media are the most influential

Table 6. Correlation matrix for total lethality, initial sample temperature (Initial T) and initial heating medium (CannerT).

	Total F	**Initial T**	**Canner T**
Total F	1.00	0.77	0.46
Initial Temperature	0.77	1.00	0.21
Canner Temperature	0.46	0.21	1.00
Heating Rate	-0.64	-0.48	-0.60
Cooling Rate	0.41	0.04	0.48

parameters in the final lethality of the process. The heating rate appears to have a negative correlation with the final lethality of the samples; however, this can be explained by the temperature reduction of the samples while the heating medium reached the required process temperature.

From these analyses, Initial T (Initial Temperature) and cooling rate are identified as the most significant factors influencing Total F. Canner T (Canner Temperature) also shows a positive effect, but it is not consistently significant across all models. Heating rate does not significantly influence Total lethality.

3.3 Brix Degrees and pH

The results of the pH and brix degrees measurements (Table 7) indicate that there is no significant variance between the samples ($P<0.05$). All the samples analyzed where in the required values for brix (>60) and pH (<4.6). This means that the samples are appropriate for being canned under the recommendations of high-acid foods without the need for involving pressure in the cooking process. Additionally, the samples can be considered jams, due to the dissolved solids contents.

Table 7. Descriptive summary: Brix degrees and pH

	Brix	pH
Count	30	30
mean	64.4	3.43
std	2.43	0.074
min	60	3.31
max	68	3.59

3.4 Antioxidant Activity

It is known that the thermal processing and storage of foods rich in antioxidants affect the total content of antioxidant compounds present in the food due to degradation of the compounds (Wilkes et al., 2013). Despite the reduction of the antioxidant compounds observed in this study (Table 8), the food can retain the antioxidant capacity due to the synthesis of new chemical compounds that retain the ability to scavenge free radicals (Oniszczuk et al., 2019).

Table 8. Change in antioxidant activity due to processing

Step	Antioxidant Activity (%)	Change
Fresh	91	NaN
Cooked	84	-7
Jam	79	-5
Stored	78	-1

NaN: Not Applicable

The most important process involved in the decomposition of the antioxidants is when cooking the fresh chokeberries, having the most significant effect on the compounds of interest ($P = 0.00013$), followed by the canning of the fruit ($P=0.0012$) and storing the jam for a month has the most negligible effect on the loss of nutrients ($P = 0.0007$). From this information, it can be said that even if there is a decrease in the antioxidant capacity, the properties of the food are highly maintained, and the health benefits can still be obtained from the finished product.

3.5 Shelf-Life Studies

After one month, the samples were plated on petri films for aerobic plate counts (APC) and yeast and mold (YM) counts. All the samples were tested after two weeks and four weeks and had counts below the detection limit of the petri films (<10). The results indicate that the thermal process done to the jams effectively reduced the microbial loads enough to render the food safe for home-canning applications.

4. CONCLUSIONS

The thermal processing of acid foods can create delicious and safe recipes for *Aronia* berries jams. The studies carried out demonstrated that the food, when processed for 15 minutes in boiling water canning, will reach thermal lethality ranging from 1773 min to 11058 minutes. It is important to note that the initial temperature of the jam before canning and the temperature of the heating medium where the canning process will be done is crucial to the overall lethality obtained. The process has well surpassed the requirements and can be said to be safe for household applications. All the samples tested had adequate sugar levels, and the pH of the samples was in the range of acid foods. The antioxidant activity of the samples was affected by the thermal processing of the food and by the storage at room temperature. However, this desirable characteristic is still valuable in accessing the potential benefits of the *Aronia melanocarpa* fruit. Furthermore, the microbiological analysis of the samples after one month of storage revealed that the process was sufficiently effective in controlling any growth of the microorganisms. According to the overall results of this study, the adaptation of the jam recipe from the National Center for Home Food Preservation was successful, making it a potentially helpful recipe for households who desire to have a delicious yet safe option to store and process their chokeberries. The people who desire to create a delicious *Aronia* berry jam are

recommended to follow the steps described in section 2.1 of this document. The recommended recipe consists of 2268 grams of *Aronia* berries and 1651 grams of sugar, with 93 grams of pectin for gel stability.

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Chapter 3. SALSA RECIPE DEVELOPMENT AND VALIDATION WITH *ARONIA* BERRY AS THE PRIMARY INGREDIENT, ENSURING ITS MICROBIOLOGICAL SAFETY AND QUALITY THROUGH APPROPRIATE THERMAL TREATMENT

ABSTRACT

This study aimed to develop and validate a safe and high-quality home-canned spicy salsa recipe incorporating *Aronia* berries, according to the USDA recommendations for home-canning recipes. Using a Box-Wilson Circumscribed Central Composite Design, nine salsa recipes were formulated with varying quantities of *Aronia* and onion. Sensory evaluations by untrained panelists assessed flavor, acidity, hotness, aftertaste, and overall liking. The optimal recipe was determined using response surface methodology and thermal lethality studies to ensure microbiological safety. The results indicated that the heating and cooling phases significantly contributed to the overall lethality, with moderate correlations between initial temperatures and total lethality. This research provides a comprehensive and thorough approach to developing home-canned salsa recipes that meet sensory and safety standards.

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1. INTRODUCTION

The popularity of home-canned foods is increasing due to a growing interest in sustainable living, local food movements, and the desire for homemade, preservative-free products. However, it is crucial to ensure the safety of these home-canned goods, as improper canning techniques can pose serious health risks, such as botulism (Anderson & Zhao, 2021). The USDA guidelines play a crucial role in helping home canners safely preserve foods (USDA, 2009). This study emphasizes the importance of following these safety standards, as confirmed by our research, to safeguard the health and well-being of consumers. The significance of this issue and the necessity for ongoing research to validate new recipes that not only comply with these safety standards but also consider and accommodate consumer preferences are evident.

Aronia berries, also known as chokeberries, are gaining popularity due to their high antioxidant content and potential health benefits, as highlighted in numerous studies (King et al., 2020; Rahmani et al., 2019; Sidor et al., 2019; Mahoney et al., 2019). This research focuses on the unique opportunity of integrating *Aronia* berries into traditional recipes, such as salsa, to add nutritional value and offer a unique flavor profile that could appeal to a broad audience. The study aims to develop a spicy salsa recipe with *Aronia* berries as the primary ingredient, ensuring its microbiological safety and quality through appropriate thermal treatment. Incorporating these berries enriches the culinary experience and provides potential health benefits to consumers, making it an attractive option for health-conscious individuals.

The research objectives were twofold: to develop a recipe that balances sensory attributes such as flavor, acidity, hotness, aftertaste, and overall liking and to validate the

recipe's safety through thermal lethality studies. The study employed a Box-Wilson Circumscribed Central Composite Design to systematically vary the quantities of *Aronia* berries and onions in the recipes. Sensory evaluations were conducted with untrained panelists, and response surface methodology was used to determine the optimal recipe.

Thermal lethality studies were conducted to ensure microbiological safety. These studies measured the lethality during the heating and cooling phases of the canning process, using thermocouples to monitor temperatures and calculate the lethality values. The total lethality was assessed to ensure it met the required standards for safe home canning.

The initial temperatures of the salsa samples and the canner were also investigated for their impact on total lethality. This comprehensive approach provides valuable insights into developing home-canned products that are both safe and appealing to consumers.

2. MATERIALS AND METHODS

2.1. Spicy *Aronia* Salsa Preparation

Canning jars (16 fl oz, 473 mL., Ball Canning Corporation, Muncie, IN) were sterilized, and the two-piece canning lids were prepared according to the manufacturer's instructions, which were put into boiling water for ten minutes before filling. Onion, finely chopped serrano peppers, water, cider vinegar, canning salt, sugar, and clover honey were combined into a large saucepan and brought to a boil over high heat; the heat was slightly reduced, and the mixture was boiled for five minutes. The chokeberries were added to the saucepan, the heat was reduced, and the salsa was simmered for twenty

minutes with constant stirring to avoid scorching the mixture. The salsa was immediately placed in the sterile jars, leaving ¼ inch of headspace, and the visible bubbles in the jar were removed. The rims of the jars were wiped with a clean, damped paper towel, and the two-piece metal canning lids were closed slightly tight. The jars were placed in a 23-liter boiling water canner (BWC) (Victorio VKP1130; Victorio Kitchen Products, Orem, UT) and processed for 15 minutes. The cooked jars were removed from the BWC and let cool undisturbed overnight. The seals were checked to ensure all the jars were airtight.

2.2. Experimental Design and Optimization

Nine salsa recipes were developed (Table 2) using a Box-Wilson Circumscribed Central Composite Desing, with an alpha of 1.414 appropriate for systems with two factors affecting the analysis's response, in this case, *Aronia* and onion, as shown in Table

Factor	Parameter	Low Limit (g)	High Limit (g)
A	Amount of *Aronia*	1480	1880
B	Amount of Onion	600	960

Table 2. Combination of *Aronia* Salsa formulation with coded variable levels for experimental design

Recipe	Amount of *Aronia*, g (X_1)	Onion, g (X_2)
1	1480 (-1)	600 (-1)
2	1880 (1)	600 (-1)
3	1480 (-1)	960 (1)
4	1880 (1)	960 (1)
5	1680 (0)	780 (0)
6	1680 (0)	780 (0)
7	1507 (-1.414)	780 (0)
8	1853 (1.414)	780 (0)
9	1680 (0)	625 (-1.414)
10	1680 (0)	935 (1.414)
11	1680 (0)	780 (0)
12	1680 (0)	780 (0)

2.3. Sensory Evaluation

Thirty untrained sensory panelists were recruited for the *Aronia* and thirty-one for the salsa. For each product, nine random numbers were assigned to each recipe to avoid biases from the panelists. The panelists entered a booth where they received nine samples, one at a time. The panelists had to evaluate the products of 5 different parameters on a 9-point hedonic scale: flavor, acidity, hotness, aftertaste, and overall likeness of the salsa. The results from the sensory were evaluated and fitted to a quadratic model to estimate the best combination of onion and *Aronia*. The models were evaluated using ANOVA to ensure their goodness of fit. The IRB project # for this part of the research was 23535.

2.4. Thermal Lethality Studies

Once the sensory evaluation data was evaluated an optimized recipe was created with the model equations and this recipe was used for thermal lethality studies. Two-piece lids were modified by perforating a hole in the center of the lid, and stuffing boxes

(Pouch receptacle C5.2; TechniCAL, New Orleans, LA) were fitted to the lids. The thermocouples (Temperature distribution/Free lead wires; TechniCAL, New Orleans, LA) were inserted into the stuffing boxes in a manner that the measuring tip of the thermocouple was in the geometrical center of the jars (16 fl oz, 473 mL; PT), around 7 centimeters from the top. The salsa was cooked following the procedures explained in section 2.1 of this document, replacing the two-piece lids with the modified lids. Once the jars were filled and the modified lids adjusted, the thermocouples were connected to a data logger (CALPlex Datalogger; TechniCAL, New Orleans, LA) equipped with Calsoft 6 software (TechniCAL, New Orleans, LA). The data jars were placed in a boiling water canner with preheated water at 82°C. When the water reached boiling point, the cooking time started, and the product was held in the BWC for fifteen minutes according to USDA recommendations. The temperatures were recorded by the software every minute, and the heating and cooling lethality of the process was calculated using the software, which implemented the ball formula method. The total lethality of the process was calculated by solving the integral of the ball formula with the trapezoidal method.

2.5. Shelf-life Studies

For the shelf-life study, the jars from each run of the optimized salsa recipe were stored at room temperature (20-25°C) for one month. Once the month passed, the canned jam and salsa were evaluated for aerobic plate count with dilutions 10^{-1} to 10^{-3} using yeast and mold (YM) Petrifilm and aerobic plate counts (APC) Petrifilm (3M; Saint Paul, MN).

3. RESULTS AND DISCUSSION

3.1. Model Fitting and Analysis of Variance

Table 3. Mean sensory analysis data for the sensory evaluation of *Aronia* berry salsa

Run	Factors		Responses				
	Aronia content (g)	Onion content (g)	Flavor	Acidity	Hot(pepper)	Aftertaste	Overall liking
1	1480	600	5.24	5.32	6.65	5.38	5.38
2	1880	600	5.65	4.68	6.44	5.44	5.62
3	1480	960	4.85	4.82	6.44	5.03	4.97
4	1880	960	5.06	4.94	6.62	5.35	5.06
5	1680	780	4.74	5.03	6.47	5.03	4.71
6	1680	780	4.72	5.11	6.49	5.1	4.81
7	1507	780	5.87	5.35	6.77	5.65	5.65
8	1853	780	5.97	4.84	6.52	5.48	5.55
9	1680	625	5.87	5.03	6.77	5.45	5.61
10	1680	935	5.9	5.26	6.87	5.87	5.71
11	1680	780	4.78	5.09	6.5	5.05	4.79
12	1680	780	4.74	5.02	6.45	5.03	4.72

This project was started to develop safe, delicious recipes for home-canned spicy *Aronia* salsa. To achieve this, surface response methodology with a circumscribed central composite design was used to create different recipes that could account for variations in the sensorial perception of different palates. Table 3 shows the mean response values for sensory analysis of chokeberry salsas.

From Table 3, it can be observed that the most liked attribute of the salsa was the hotness of the sample, having a mean of 6.58, followed by the aftertaste, with a mean of 5.32 (Table 3). Run # 10, done with recipe #5, had the highest overall liking, with a mean

of 5.71. Each parameter was fitted to a quadratic model with interactions; this model includes linear terms of the predictors and the quadratic terms, allowing the investigation of non-linear relations between the factors affecting the responses.

The salsa samples were generally found to have good acidity, with some panelists enjoying the mild heat levels. However, there were consistent concerns about the flavor profile. Many panelists felt that the salsas lacked a distinct flavor beyond the aronia berries, with some describing the taste as bland or one-dimensional. The sweetness levels were often critiqued, with several comments noting that the salsas were either too sweet or not balanced enough with acidity. The texture also received mixed reviews; while some appreciated the consistency, others felt that the whole berries were out of place in a salsa, suggesting that they should be chopped or crushed to improve mouthfeel. Overall, while the salsas had some positive aspects, such as good acidity and a unique flavor profile, there were significant suggestions for improvement, particularly in balancing the flavors and adjusting the texture.

Additionally, the quality of the fit of each model was calculated using adjusted R-squared, another version of the R-squared that accounts for the number of factors in the model. The Adjusted R^2 is calculated with the formula:

$$AAAAAAAAAAqqSSAA\ RR^2 = 1 - \left[\frac{\left[1-RR^1\right](AA-1)}{AA-kk-1}\right] \quad (2)$$

R^2 is the coefficient of determination that measures the proportion of variability in the response explained by the factors, n is the number of observations, and k is the number of factors in the model (Karch, 2020). The model equations for each response are:

$$\log(FFFFSSSSFFFF) = 1.7214 + 0.044 \cdot XX_1 + 0.1113XX_1^2 - 0.0466\,XX_2 + 0.1018XX_2^2 - 0.0452XX_1XX_2 - 0.1884XX_1^2XX_2^2 \quad (3)$$

$$\log(AASSSSAASSaaaa) = 1.6235 - 0.1015 \cdot XX_1 - 0.0287XX_1^2 + 0.021\,XX_2 - 0.0052XX_2^2 - 0.2097XX_1XX_2 - 0.1402XX_1^2XX_2^2 \quad (4)$$

$$\log(HHFFaaSSSSAAAA) = 1.8954 - 0.0312 \cdot XX_1 + 0.0166XX_1^2 + 0.0096\,XX_2 + 0.0439XX_2^2 + 0.0819XX_1XX_2 - 0.0364XX_1^2XX_2^2 \quad (5)$$

$$\log(AAAAaaSSFFaaSSAAaaSS) = 1.6793 - 0.0929 \cdot XX_1 - 0.0362XX_1^2 + 0.0322\,XX_2 + 0.1324XX_2^2 + 0.0694XX_1XX_2 - 0.2421XX_1^2XX_2^2 \quad (6)$$

$$\log(OOSSSSFFSSFFFF) = 1.6907 - 0.01 \cdot XX_1 + 0.0769XX_1^2 - 0.0416\,XX_2 + 0.1297XX_2^2 - 0.0353XX_1XX_2 - 0.1971XX_1^2XX_2^2 \quad (7)$$

For these equations, the adjusted R^2 values were 0.9972 for flavor, 0.9537 for acidity, 0.9975 for hotness, 0.9827 for aftertaste, and 0.9819 for the overall liking of the samples.

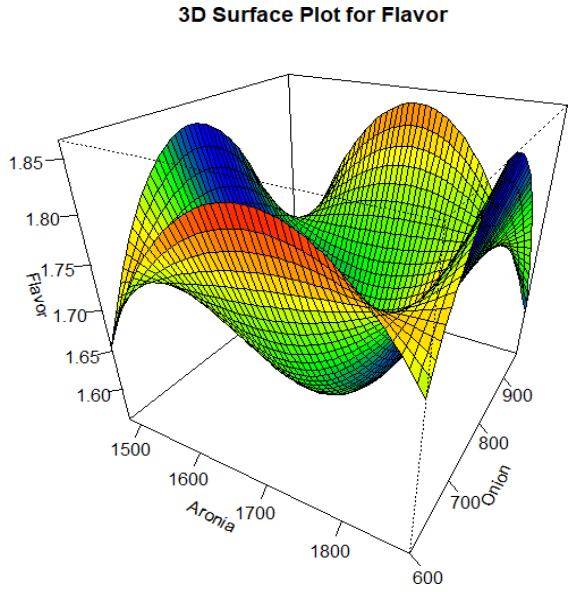

Figure 1. Surface of response of Flavor

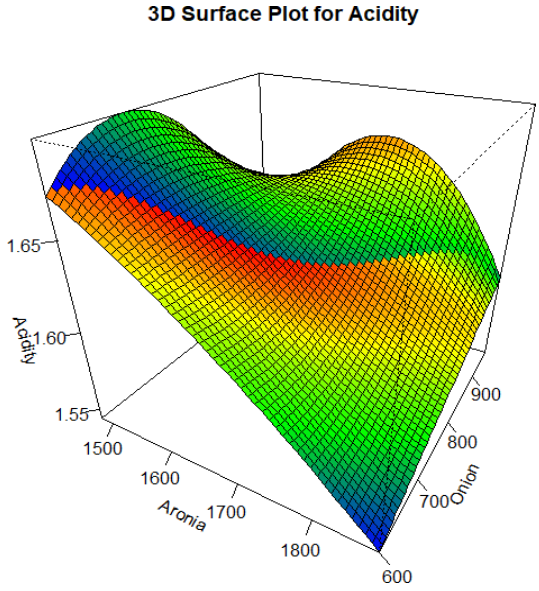

Figure 2. Surface of response of Acidity

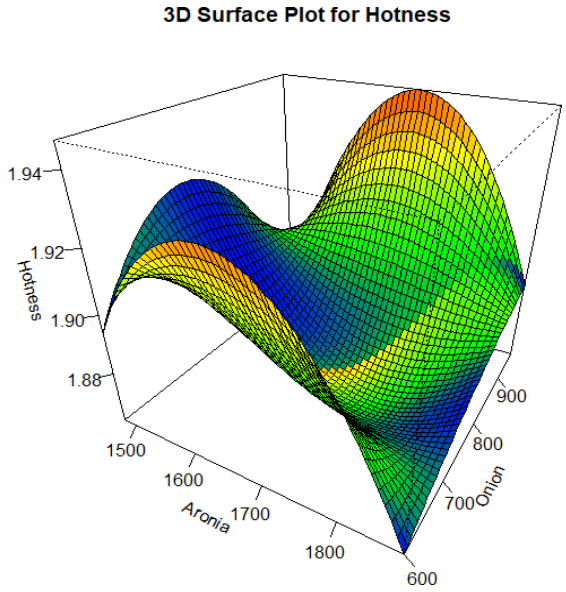

Figure 3. Surface of response of Hotness

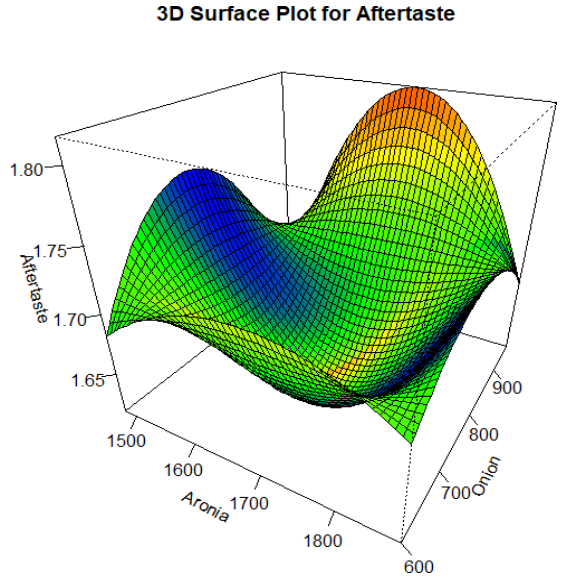

Figure 4. Surface of response of Aftertaste

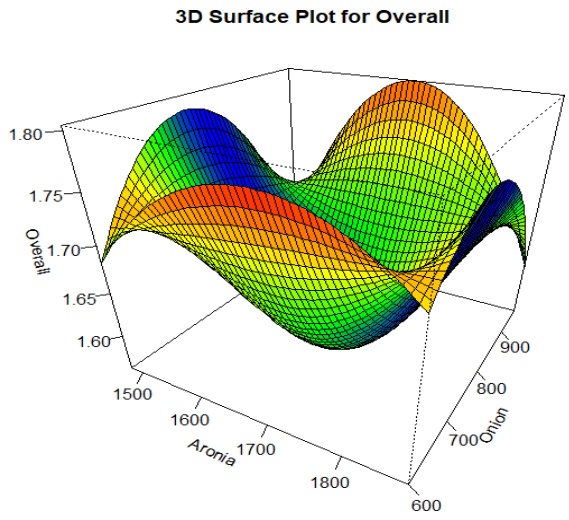

Figure 5. Surface of response of Overall liking

The equations allow for generating the surface of response graphs, which show the interactions of the quantities of *Aronia* (X_1) and onion (X_2). The surface of the response in Figures One to Five shows which combinations of the factors have better responses among the panelists that tested the samples.

The data from the graphs indicates that the highest responses occur when moderate amounts of *Aronia* are combined with both low and high amounts of onion. The ideal quantities of *Aronia* and onion to achieve the highest overall liking responses are 1686 grams and 960 grams, respectively, resulting in a predicted overall score of 6.08.

3.2. Thermal Lethality

Upon the optimization of the recipe for the spicy *Aronia* salsa, lethality studies were performed to learn if the processing of the salsa was sufficient to inactivate the bacterial communities present in the food. Previous research showed that the initial

sample temperature and initial heating medium temperature influenced the total lethality ($F_{82.2°C}$). In this study, the layout of the samples was replicated from the one in Chapter 2 of this thesis (Figure 6).

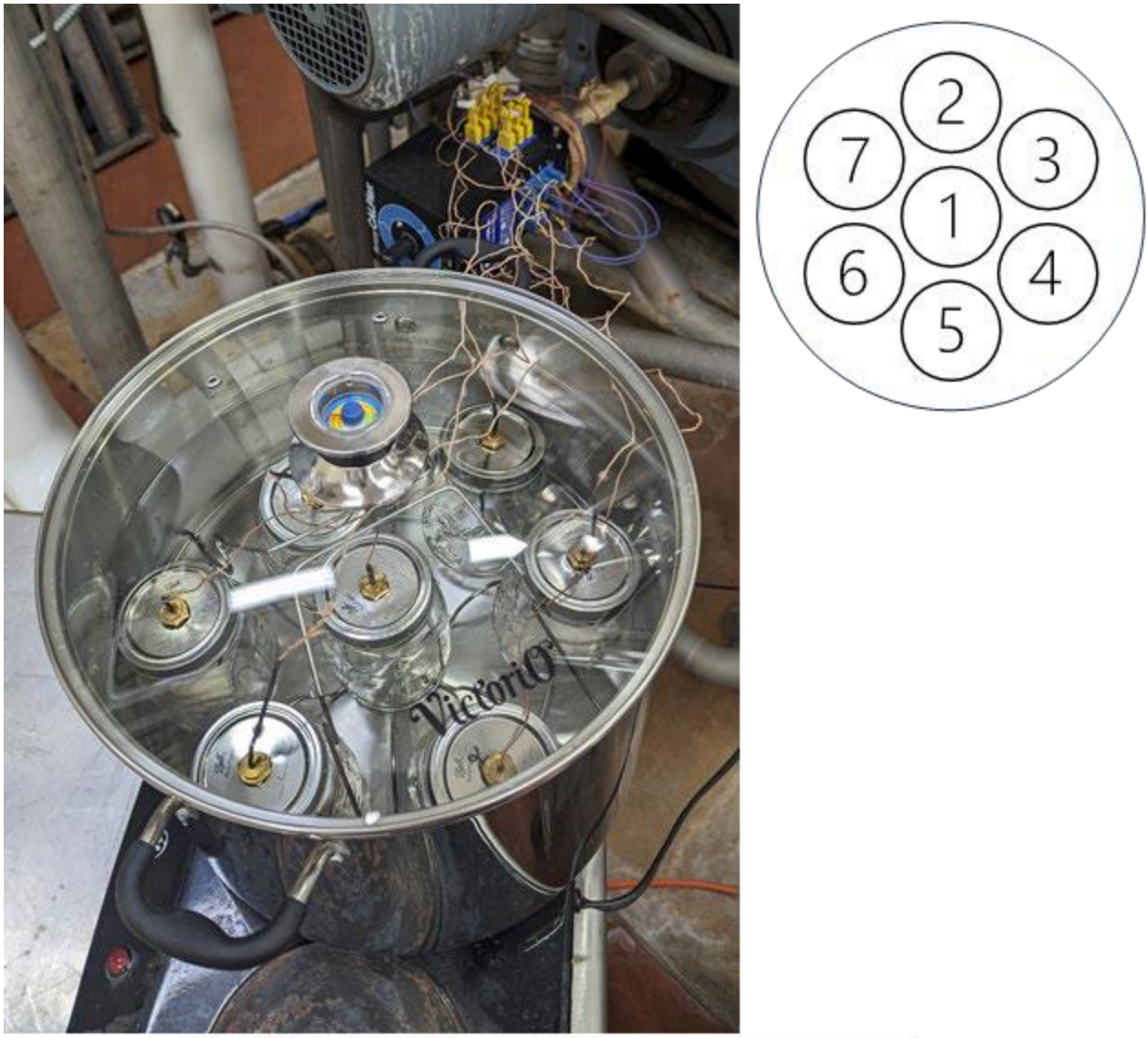

Figure 6. *Layout of Thermocouples*

The influence of the initial temperature of the samples and the heating medium was analyzed again to determine if this system's influence on the total lethality was observed.

Table 4. Summary of Analysis

Parameter	Mean	Standard Deviation	Minimum	Maximum
Rate of Heating (fh)	37.75 min	9.2 min	24 min	49 min
Rate of Cooling ($f$cl)	74 min	14.98 min	61 min	98 min
Lethality during Cooling (Fcl)	3392.03 min	1231.23 min	1161.74 min	6430.36 min
Lethality during Heating (Fh)	3445.63 min	1155.44 min	784.42 min	5840.83 min
Total Lethality (FTotal)	6837.66 min	2106.88 min	1946.16 min	12271.19 min
Initial Temperature (TInitial)	79.31°C	4.18°C	74.08°C	87.18°C

From Table 4, we can observe that the cooling rate was almost double that of heating; however, there is no statistical difference in the lethality during the cooling and heating phases of the process (P = 0.991).

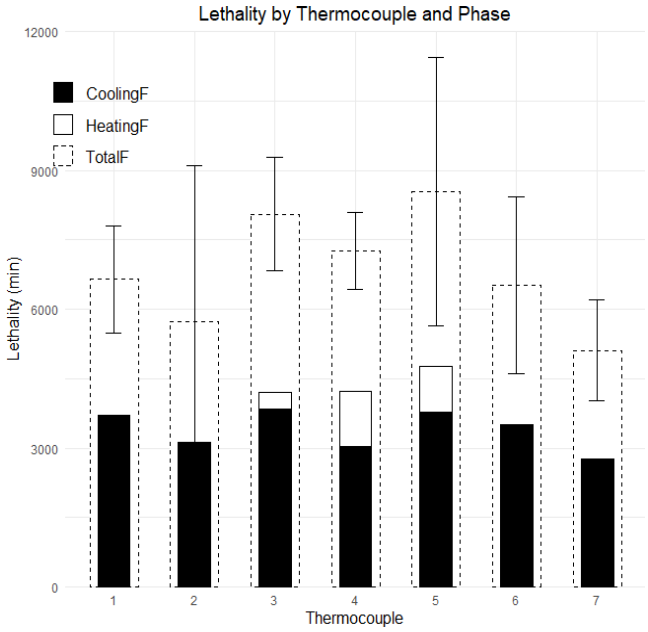

Figure 7. *Thermal Lethality by Thermocouple location and process step*

Figure 7 shows the mean $F_{82.2°C}$ (in minutes) observed at various thermocouple locations during different phases of the thermal process: Cooling $F_{Cl\text{-}82.2°C}$ (black bars), Heating $F_{H\text{-}82.2°C}$ (white bars), and Total $F_{82.2°C}$ (striped bars). The variability and distribution of lethality measurements across seven thermocouple locations can be observed. Error bars represent the standard deviation, indicating the variability within each phase at each thermocouple location. The cooling and heating phases had a very similar effect on the lethality of the processed salsas (Figure 7). Figure 8 compares the lethality (measured in minutes) across three different phases: CoolingF, HeatingF, and TotalF; each phase is represented in the box plot, showing the distribution of the lethality values. The cooling phase showed the lowest median and a slight variability in the lethality values and outliers, which were higher and lower than the rest. The heating phase showed more variability than the cooling phase but less than the total lethality of the process. Additionally, it had no

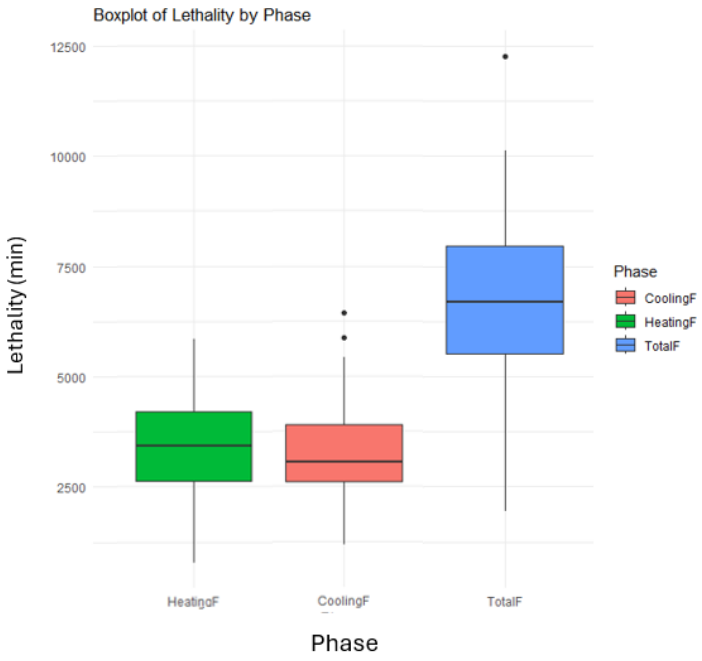

Figure 8. *Comparison of Lethality across cooling, heating and totality of the process.*

significant outliers, suggesting that most of the samples fall within a consistent range of values.

The total lethality (F_{total}) is a cumulative measure incorporating the cooling and heating lethality values. F_{total} has more variability and outliers, suggesting that the combined effects of heating and cooling phases introduce more differences in lethality across samples. The differences in median values across the phases indicate that each phase contributes differently to the overall lethality, with the cooling phase contributing the least and the total phase capturing the combined effects of both phases.

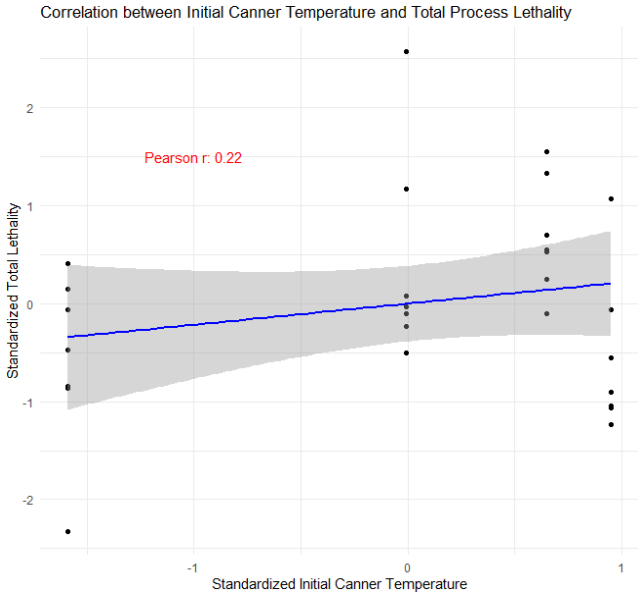

Figure 9. *Correlation between initial canner temperature and total process lethality*

Figure 9 shows a weak positive correlation (Pearson r = 0.22) between the initial canner temperature and the total process lethality. This weak correlation indicates that the initial canner temperature has a small significant impact on the total process lethality. The

wider confidence interval band around the regression line suggests more variability in the data, implying that factors other than the initial canner temperature might influence the total process lethality.

Table 5. Descriptive Statistics and Correlation with Total Lethality for Various Parameters

Parameter	Mean	Standard Deviation	Minimum	Maximum	Correlation with Total Lethality (r)
Rate of Heating (fh)	37.75 min	9.2 min	24 min	49 min	0.48
Rate of Cooling ($f$cl)	74 min	14.98 min	61 min	98 min	0.29
Lethality during Cooling (Fcl)	3392.03 min	1231.23 min	1161.74 min	6430.36 min	0.89
Lethality during Heating (Fh)	3445.63 min	1155.44 min	784.42 min	5840.83 min	0.87
Total Lethality (FTotal)	6837.66 min	2106.88 min	1946.16 min	12271.19 min	NA
Initial Temperature (TInitial)	79.31°C	4.18°C	74.08°C	87.18°C	-0.63

Table 5 provides a detailed summary of various parameters related to the lethality process, presenting descriptive statistics such as mean, standard deviation, minimum, and maximum values. It also shows the Pearson correlation coefficients between each parameter and the total lethality (F_{Total}).

The heating rate (f_h) has a mean of 37.75 minutes with a standard deviation of 9.2 minutes. The heating rates vary from a minimum of 24 minutes to a maximum of 49 minutes. The correlation between the heating rate and total lethality is 0.48, indicating a moderate positive relationship. This suggests that higher heating rates are associated with higher total lethality, implying that more rapid heating may enhance the lethality of the

process. For the cooling rate (f_{cl}), the mean value is 74 minutes with a standard deviation 14.98. The cooling rates range from 61 minutes to 98 minutes. The correlation between the cooling rate and total lethality is 0.29, indicating a weak positive relationship. This suggests that while there is some association between higher cooling rates and total lethality, it is not as strong as the relationship with the heating rate.

The lethality during cooling (F_{cl}) has a mean of 3392.03 minutes and a standard deviation of 1231.23 minutes. The values range from 1161.74 minutes to 6430.36 minutes. The correlation between lethality during cooling and total lethality is 0.89, indicating a robust positive relationship. This implies that the lethality achieved during the cooling phase significantly contributes to the overall lethality. On the other hand, the lethality during heating (F_h) has a mean of 3445.63 minutes with a standard deviation of 1155.44 minutes, ranging from 784.42 minutes to 5840.83 minutes. The correlation with total lethality is 0.87, a strong positive relationship. This suggests that the lethality achieved during the heating phase is crucial for the overall lethality of the process. The total lethality (F_{Total}) has a mean of 6837.66 minutes and a standard deviation of 2106.88 minutes, with values ranging from 1946.16 minutes to 12271.19 minutes.

Lastly, the initial temperature ($T_{Initial}$) has a mean of 79.31°C and a standard deviation of 4.18°C, ranging from 74.08°C to 87.18°C. The initial temperature and total lethality correlation is -0.63, indicating a moderate negative relationship. This suggests that higher initial temperatures are associated with lower total lethality, which might be because higher initial temperatures may lead to faster achievement of lethal conditions, reducing the overall time required for the process.

Table 6 highlights the importance of both heating and cooling rates in influencing the total lethality of the process. The lethality during the cooling and heating phases shows the strongest correlations with total lethality. The initial temperature also plays a significant role, though negatively.

3.3. Shelf-life Studies

To determine the shelf-life of the salsa, the samples were tested every two weeks in one month, looking at aerobic plate counts (APC) and yeast and molds (YM). For each test, the counts were below the detection limit of the petri films (<10). These results show that the processing of the salsa was sufficient to reduce the presence of microorganisms, creating safe products for home canning.

4. CONCLUSIONS

Developing a spicy *Aronia* salsa involved optimizing the balance between *Aronia* berries and onions to achieve the best sensory appeal. Using a Box-Wilson Circumscribed Central Composite Design allowed for a comprehensive analysis of the sensory attributes, resulting in a preferred recipe with high overall liking. The optimized recipe contains 1686 grams of *Aronia* berries and 960 grams of onions, prepared as described in section 2.1 of this document.

Thermal lethality studies confirmed the safety of the canning process, highlighting the significant contributions of both heating and cooling phases to the total lethality. Processing the salsa for 15 minutes achieved a thermal lethality from 1946 min to 12271 min, which translates to logarithmic reductions of $2.4x10^7$ to $1.5x10^9$ CFU/g. surpassing the 5-log reductions of *Escherichia coli*.

The correlations between initial temperatures and lethality suggest that optimizing these parameters can enhance the safety and efficiency of the canning process. Specifically, higher initial temperatures of the samples correlated with lower total lethality, indicating that faster achievement of lethal conditions may reduce the overall process time.

This study provides a robust framework for developing home-canned products that are both safe and appealing to consumers. The research offers a model for future recipe development and validation in home canning by integrating thorough sensory evaluations with rigorous safety assessments. The successful incorporation of *Aronia* berries into a popular food product like salsa adds nutritional benefits and supports the diversification of home-canned foods available to consumers.

5. FUTURE WORK

To fully assess the stability and safety of Aronia-based products, it is crucial to conduct extended shelf-life studies. These studies should include regular monitoring of pH, water activity (aw), aerobic plate counts (APC), yeast and mold counts (YM), and antioxidant levels. Sensory evaluations at different intervals (e.g., 0, 3, 6, 9, and 12 months) will provide insights into how the products evolve over time in terms of taste, texture, and overall consumer acceptability. Aronia berries are known for their high levels of antioxidants and bioactive compounds, which may possess antimicrobial properties. Future research could explore the potential of Aronia extracts as natural preservatives in food products. This could involve testing the antimicrobial efficacy of Aronia against common foodborne pathogens and spoilage organisms, both in isolation and as part of food formulations.

Building on the success of the current recipes, new product formulations incorporating Aronia could be developed. This could include fermented products like kombucha, Aronia-based beverages (juices, smoothies, beers), and other functional foods that leverage the health benefits of Aronia. Exploring different flavor pairings and processing methods could help create products that appeal to a broader audience. Based on the feedback from sensory evaluations, future research should focus on optimizing the sensory qualities of Aronia-based products. This could involve refining the texture, sweetness, and acidity balance to meet consumer preferences better. The use of different sweeteners, acids, or texture modifiers could be tested to enhance the overall sensory experience. Developing educational materials and resources, such as a NebGuide and a dedicated webpage, could help raise awareness about the benefits of Aronia berries and how to incorporate them into daily diets. This could include recipe guides, health benefits, and tips on using Aronia in various culinary applications. Engaging with consumers through online platforms and social media could further promote these products.

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