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## The Effect of Different Ethanol Concentrations on Functional Properties in Apple Macerates

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### ABSTRACT

**Background:** Apples are globally recognized for their nutritional value and being rich in bioactive compounds like polyphenols, which are associated with numerous health benefits, including reduced oxidative stress and lower risks of chronic diseases. This study investigates the impact of varying ethanol concentrations (20%, 40%, and 70%) on the extraction of polyphenols, vitamin C, and sugars from Jonagold apples (*Malus domestica*). **Methods:** The experiment involved macerating apple cubes in different ethanol concentrations for 30 days at 22 °C, followed by chemical analyses to quantify the extracted polyphenols, vitamin C, and sugars. **Results:** The highest polyphenol concentration (69.99 mg/100 g fresh weight) and sugar (0.936 g/100 g fresh weight) were found in the 40% ethanol macerate, while the highest vitamin C (0.043 mg/100 g fresh weight) contents was observed in the 20% ethanol macerate. The efficiency of extraction varied significantly with ethanol concentration, with the 40% ethanol solution being most effective for polyphenols and sugars and 20% for vitamin C and sugars. **Conclusion:** These findings highlight the importance of optimizing solvent concentration to maximize the extraction of specific bioactive compounds, offering valuable insights for the production of apple-based alcoholic beverages and nutraceuticals. The study underscores complex interactions between solvent polarity and compound solubility, which are crucial for enhancing the nutritional and therapeutic potential of apple-derived products.

### Introduction

The apple (*Malus domestica*) is one of the most widely consumed fruits globally, renowned for its versatility, nutritional value, and the extensive variety of cultivars available. Apples occupy a prominent position in global diet,

consistently ranking among the top fruits produced and consumed worldwide. According to the Food and Agriculture Organization (FAO), the projected annual production of apples for 2023-2024 is expected to exceed 83.7 million metric tons (Zang,

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2024). Apple consumption extends beyond fresh fruit to a range of processed forms, including juices, sauces, and dried products. This widespread consumption is attributed to their palatability, convenience, and the diversity of apple cultivars, each offering distinct flavors, textures, and culinary applications (Maragò *et al.*, 2016, Vidović *et al.*, 2020). The importance of apples as a dietary staple has spurred extensive research into their nutritional composition and the associated health benefits. Among the various bioactive compounds present in apples, polyphenols, vitamin C, and sugars have garnered considerable attention.

Polyphenols, a diverse group of naturally occurring compounds, are central to the health benefits attributed to apples. Numerous studies have demonstrated the role of apple polyphenols in reducing oxidative stress, thereby mitigating the risk of chronic conditions such as cardiovascular disease, diabetes, and certain cancers (Boyer and Liu, 2004, Scalbert *et al.*, 2005, Yeung *et al.*, 2021). Flavonoids such as quercetin, epicatechin, and procyanidins are particularly abundant in apples, each contributing uniquely to human health (Mushtaq *et al.*, 2020).

Ascorbic acid (vitamin C), an essential nutrient found in significant quantities in apples, plays a critical role in various physiological processes, including collagen synthesis, immune function, and antioxidant activity (Steinberg, 2022). As humans cannot synthesize vitamin C, it must be obtained from dietary sources (Aburawi, 2021, Carr and Maggini, 2017). Although apples are not as rich in vitamin C as citrus fruits, their contribution to dietary vitamin C intake remains significant due to their widespread consumption (Lee and Kader, 2000). The synergistic effects of vitamin C and polyphenols in apples enhance the overall antioxidant capacity of the fruit, providing robust protection against oxidative damage (Manach *et al.*, 2004).

The sugar content in apples, predominantly fructose, glucose, and sucrose, plays a key role in both their nutritional value and sensory properties. Sugars are a primary energy source, and their natural presence in fruits like apples is preferable

to added sugars in processed foods due to the accompanying fiber and phytonutrients that modulate glucose absorption and metabolism (Ludwig, 2002). The balance and concentration of these sugars vary across apple cultivars, influencing the fruit's sweetness, flavor profile, and glycemic index. Typically, apples contain approximately 10-15 grams of sugars per 100 grams of fresh weight, classifying them as a moderate glycemic index fruit (Wojdyło *et al.*, 2008). The natural sugars in apples provide a quick energy source while being moderated by fiber, which regulates absorption rates and impacts glycemic responses (Giuntini *et al.*, 2022, Paquet *et al.*, 2014, Schulze *et al.*, 2004).

In recent years, there has been a growing interest in alcoholic beverages derived from apples, such as cider and apple brandy (Calvert *et al.*, 2024). These beverages not only offer unique flavors but also retain some of the nutritional benefits of fresh apples. The fermentation process can alter the polyphenolic profile, potentially enhancing the bioavailability of certain compounds (Dávalos *et al.*, 2005). Additionally, alcoholic extracts of apples have been studied for their antimicrobial properties and potential health benefits, adding a new dimension to the utilization of this versatile fruit (Kara *et al.*, 2021). However, the efficiency of the extraction process and the content of the obtained solution are influenced by factors such as the solvent type, solvent-to-plant ratio, plant part/size used, extraction time, temperature, and the maceration container (Plaskova and Mlcek, 2023).

The existing body of literature primarily focuses on fermented alcoholic apple beverages, leaving a notable gap in research on non-fermented alcoholic apple beverages (Lin *et al.*, 2025, Tsoupras *et al.*, 2023). Only a limited number of studies have explored how varying alcohol concentrations influence selected nutritional parameters of apples (López *et al.*, 2017). In the present study, the authors aim to address this research gap by conducting an extensive analysis of health-promoting constituents in alcoholic apple macerates. Specifically, the study examines the

polyphenol content, vitamin C levels, and sugar composition of apple macerates prepared with alcohol concentrations of 20%, 40%, and 70%.

## Materials and Methods

### Plants

Jonagold apples were cultivated in Chrzęszczew, Poland (51°50'02"N, 20°26'05"E) during the 2023 growing season.

### Preparation of macerates

One kilogram of apples was washed under tap water and dried. The cores were removed, and the fruit was cut manually into cubes of approximately 2×2 cm. The apple cubes were placed in glass jars. Three different concentrations of ethanol macerates (20%, 40%, and 70%) were prepared, corresponding to the alcohol content of common fruit-based alcoholic beverages (cocktails/liqueurs, vodka, and spirits). One liter of HPLC-grade ethanol (of appropriate concentration) was added to the fruit cubes (1 kg), and the jars were stored in the dark for 30 days at 22 °C. The time interval between cutting the apples and pouring them with ethanol of the appropriate concentration was less than 20 minutes.

### Chemical analysis

**Polyphenol content:** A portion of the fruit cubes used for maceration was transferred to a homogenizer. Five grams of fruit pulp were extracted with 20 ml of methanol (HPLC-grade, ChemPur) and filtered (rapid-filtration filter papers) into a 50 ml volumetric flask. One milliliter of the extract was mixed with 0.5 ml of Folin-Ciocalteu reagent (Sigma-Aldrich) and 1 ml of NaCO<sub>3</sub> solution (p.a. grade, ChemPur), and the volume was adjusted to 5 ml with distilled water. The samples were vortexed, incubated in the dark at room temperature for 25 minutes, and then centrifuged at 7,000 rpm, for 5 minutes. Absorbance was measured with the spectrophotometer (UV-Vis DR 5000, Lange) at 765 nm against a blank, following the method described by AOAC (Association of the Analytical Chemists (AOAC), 1974).

**Vitamin C analysis:** Vitamin C (the sum of L-

dehydroascorbic acid and L-ascorbic acid) was determined using the Tillmans method. This redox titration method relies on reducing the properties of ascorbic acid. The oxidizing agent, 2, 6-dichlorophenolindophenol (DCPIP), is reduced by ascorbic acid, transitioning from its colored oxidized form to a colorless reduced form. The titration endpoint is marked by a persistent pink color, indicating that all the ascorbic acid has been oxidized. The amount of DCPIP consumed during the reaction is proportional to the vitamin C content in the sample, facilitating quantitative analysis.

### Sugars, Total Soluble Solids (TSS), and pH:

Sugars were quantified using Bertrand's method (Lati *et al.*, 2017). This method involves the reduction of copper (II) sulfate by reducing sugars (e.g., glucose, fructose) in an alkaline solution, resulting in the formation of insoluble copper (I) oxide, a red precipitate. The amount of copper (I) oxide formed was determined via titration with a standardized potassium permanganate solution, with the quantity of reducing sugars being directly proportional to the amount of copper reduced.

TSS were measured using a portable refractometer (PCE-DRC 2). The refractometer measures the refractive index of a liquid sample, which correlates with its sugar concentration. TSS is expressed in degrees Brix, representing the percentage of dissolved solids, predominantly sugars, in the sample. However, TSS encompasses all dissolved solids, including organic acids, salts, proteins, and other substances in addition to sugars.

The pH of the samples was measured using a pH meter (Testo 206-pH), which determines the concentration of hydrogen ions in the solution, reflecting its acidity or alkalinity.

### Data analysis

The obtained results were subjected to one-way ANOVA to test the single effect of alcohol concentration in macerates on biochemical components in the solution. Kelus-Newman test at a significance level of  $\alpha=0.05$  was used for detailed analyses of mean values by isolating statistically

homogeneous groups. Results of biochemical analyses are presented as mean values plus standard deviation. Calculations were performed using Statistica 9.1 (StatSoft Polska, Kraków, Poland)

## Results

The extraction using 40% ethanol yielded the highest concentration of polyphenols ( $69.99 \pm 4.57$  mg/100 g fresh weight), which was significantly greater than that obtained with both 20% ethanol ( $47.59 \pm 2.51$  mg/100 g fresh weight) and 70% ethanol ( $46.61 \pm 2.69$  mg/100 g FW, **Table 1**). Statistical analysis indicated no significant difference in polyphenol content between 20% and 70% ethanol macerates ( $P=0.05$ ), as denoted by the same letter annotations. These results suggest that ethanol concentration plays a crucial role in the efficiency of polyphenol extraction, with 40% ethanol proving most effective in retaining polyphenols.

The highest concentration of vitamin C was observed in the 20% ethanol macerate ( $0.043 \pm 0.01$  mg/100 g FW), while the 40% and 70% ethanol macerates exhibited similarly lower levels ( $0.022 \pm 0.01$  mg/100 g FW and  $0.025 \pm 0.01$  mg/100 g FW, respectively). Statistical analysis revealed no significant difference in vitamin C content between the 40% and 70% ethanol macerates ( $P=0.05$ ). These findings indicated that ethanol extraction substantially reduces vitamin C content, with no significant variation between the 40% and 70% ethanol macerates.

Regarding sugar content, the 40% ethanol extract contained the highest amount ( $0.939 \pm 0.05$  g/100 g FW), followed by the 20% ethanol macerate ( $0.905 \pm 0.01$  g/100 g FW), while the 70% ethanol macerate had the lowest sugar content ( $0.806 \pm 0.04$  g/100 g FW). Statistical analysis showed no significant difference in sugar content between the 20% and 40% ethanol macerates

( $P=0.05$ ). These results underscore a significant reduction in sugar content during ethanol extraction, with 70% ethanol being the least effective in preserving sugar levels.

It is worth noting that, for polyphenols, vitamin C, and sugars, the differences between non-significant values varied only slightly. These differences ranged from as little as 1% for polyphenols, 7% for vitamin C, to 11% for sugar content.

The pH of the macerates increased with rising alcohol concentration. The 70% ethanol extract exhibited the highest pH (4.35), followed by the 40% ethanol macerate (4.15), and the 20% ethanol macerate (4.10). A similar trend was observed for TSS (expressed as %Brix), where the 70% ethanol extract had the highest %Brix value (14.6%), the 40% ethanol macerate exhibited a lower TSS (11.9% Brix), and the 20% ethanol macerate had the lowest TSS value (9.4% Brix).

These findings suggest that ethanol extraction influences both pH and TSS, with higher ethanol concentrations (70%) being more effective in maintaining TSS values closer to those found in fresh apples (14.6% Brix). Notably, the pH values in all macerates were higher compared to fresh apples.

As shown in the accompanying figure (**Figure 1**), the macerates exhibited variation in color intensity, with the 20% ethanol extract being the lightest, and the 70% ethanol extract being the darkest. The means in columns followed by the same letters are not significantly different at  $P=0.05$  (Kelus-Newman test).

The polyphenol content in alcoholic apple macerates varied with ethanol concentration. Higher alcohol concentrations generally enhance polyphenol solubility, leading to more efficient extraction (**Figure 2**).



Table 1. Selected chemical parameters in apples and alcoholic apple macerates.

Variable	Polyphenols (mg/100 g FW)	Vitamin C (mg/100 g FW)	Reducing sugars (g/100 g FW)	pH	TSS [%Brix]
Apple	230.87±26.25	11.55±1.18	5.99±0.15	3.64	14.6
Macerates					
20%	47.59±2.51bc	0.043±0.01a	0.905±0.01ab	4.10	9.4
40%	69.99±4.57a	0.022±0.00bc	0.939±0.05ab	4.14	11.9
70%	46.61±2.69bc	0.025±0.00bc	0.806±0.04c	4.35	14.6

TSS: Total soluble solids; FW: Fresh weight; The a,b,c mean in columns followed by the same letters are not significantly different at  $P=0.05$ .

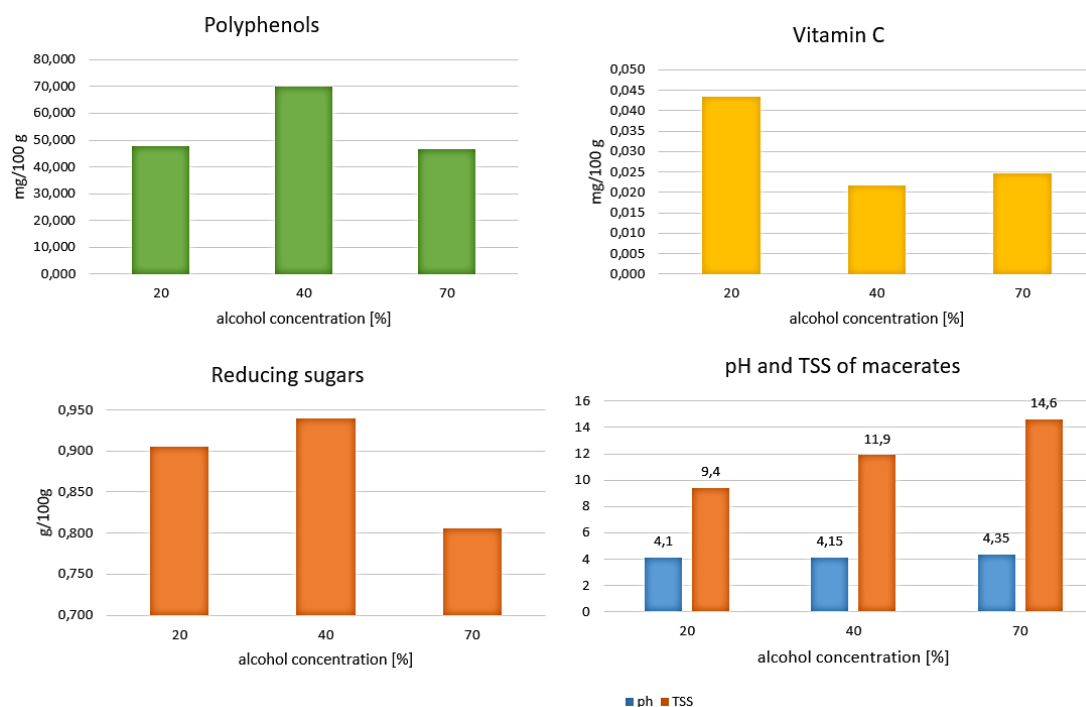


Figure 1. Colors of obtained macerates.

## Discussion

The polyphenol content in alcoholic apple macerates varied with ethanol concentration. Previous studies have demonstrated that ethanol concentrations between 40% and 60% enhance total polyphenol extraction from grape seeds (Shi *et al.*, 2003). Wang *et al.* and Pollini *et al.* reported optimal polyphenol extraction from apple pomace at ethanol concentrations of around 50% (Pollini *et al.*, 2021, Wang *et al.*, 2018). Opris *et al.* found that 50% ethanol provided the best polyphenol extraction in apples (Opris *et al.*, 2022). Human-Castilla *et al.* considered 32.5% ethanol solution as most effective in polyphenols extraction; however, the researchers were conducting the experiment at 150 °C (Huaman-Castilla *et al.*, 2019). Zheng *et al.* observed that 70% ethanol was most effective in unripe apples (Zheng *et al.*, 2009), a finding consistent with results from other fruits like black currants (Stoica *et al.*, 2013). The impact of higher alcohol

concentration on unripe fruit may be linked to ethanol's ability to disrupt plant cell walls and solubilize polyphenols more effectively than water alone. Furthermore, different polyphenols are selectively extracted depending on solvent concentration (da Silva *et al.*, 2021). Budak *et al.* observed higher polyphenol content in cider produced with maceration (Budak *et al.*, 2015). Santos *et al.* also noted that higher ethanol concentrations promote the extraction of phenolic compounds, increasing the final extract yield (Santos *et al.*, 2017). Giacobbo *et al.* highlighted the greater efficiency of organic solvents in polyphenol extraction (Giacobbo *et al.*, 2015). The polarity of the extracted compounds and the solvent is crucial, as extraction yield is highly dependent on solvent polarity (Keddar *et al.*, 2020). Plaskova *et al.* suggested the potential benefit of enzyme pretreatment in improving polyphenol release by degrading cell walls and membranes (Plaskova and Mlcek, 2023).



**Figure 2.** Content of polyphenol, vitamin C, reducing sugars and pH, total soluble solids (TSS) in macerates.

The highest vitamin C content was found in the 20% ethanol macerate (**Figure 2**). Vitamin C, being highly soluble in water, exhibits reduced solubility in ethanol, rendering extraction efficiency inversely proportional to ethanol concentration. This behavior aligns with its hydrophilic nature, which favors polar solvents like water (Israelachvili, 2011). Rahman *et al.* observed higher vitamin C levels in 50% ethanolic extracts of *Centella asiatica* compared to pure ethanol (Rahman *et al.*, 2013). Montero-Calderon *et al.* reported a positive correlation between ethanol concentration (50%) and extraction time in orange juice (Montero-Calderon *et al.*, 2019). Katja noted increased photooxidation of vitamin C with higher ethanol concentrations in spinach extracts (Katja, 2019). Ageyeva *et al.* found lower vitamin C levels in apple-fermented beverages compared to fresh fruit, which was confirmed in this research (Ageyeva *et al.*, 2023). Abouzeid, noted that highest vitamin C levels was along with the brightest color of the sample what has found a confirmation in the discussed research (**Figure 1**) (Abouzeid, 2015).

In the macerates, the highest concentration of

reducing sugar was observed in the 40% ethanol macerate, although the difference between the 20% and 40% macerates were minimal (0.034 g/100 g, **Figure 2**). Bouchard *et al.* observed a decrease in sugars' solubility with the increase in ethanol fraction (Bouchard *et al.*, 2007). Eheart *et al.* observed an 85% ethanol solution as the most effective in apple's sugars' extraction (Eheart and Mason, 1965). However Karkacier *et al.* determined higher total sugar content in macerated samples treated with water only (in comparison with methanol extraction) (Karkacier *et al.*, 2003).

The pH of alcoholic apple macerates increased with rising ethanol concentration (**Figure 2**). Several factors could contribute to this phenomenon. First, the dilution of natural acids present in apples by higher alcohol content may contribute to an increase in the solution's pH. Alcohol, with a pH of 7.33, is more basic than many natural fruit acids; thus, the addition of alcohol can result in the alkalization of the solution. Furthermore, elevated alcohol concentrations may alter chemical equilibrium among various components within the solution, potentially leading to changes in pH. Additionally, alcohol may inhibit

enzymes responsible for organic acid production in the macerate, contributing to the observed increase in pH.

TSS content in alcoholic apple macerates increased with higher alcohol concentrations (**Figure 2**). This observation is consistent with the findings of Joshi and Shandu, who reported a similar trend in alcoholic beverages with varying ethanol contents (12%, 15%, 18%) (Joshi and Sandhu, 2000). Ethanol is an effective solvent for a wide range of organic compounds. At higher concentrations, it enhances the extraction of soluble substances, such as sugars, organic acids, phenols, and other phytochemicals, from the apple matrix. Increased ethanol concentration facilitates the breakdown of plant cell walls, allowing greater release of soluble substances into the solution. Additionally, the altered pH and chemical interactions between different components due to higher alcohol concentrations can further enhance the solubility of certain substances. Ethanol may also inhibit enzymes that degrade soluble compounds, leading to their higher concentrations in the solution. However, it is important to note that excessively high alcohol concentrations may induce the precipitation of certain substances or reduce extraction efficiency. Therefore, the optimal alcohol concentration should be carefully determined based on the specific case and desired extraction outcomes.

The existing research on alcoholic apple products mainly focuses on fermented beverages, where the nutritional composition of the final product is largely determined by fermentation conditions. This focus precludes direct comparisons between the findings of this study and those from existing literature. Furthermore, research on maceration processes has generally utilized other plant materials, such as mistletoe leaves and twigs (Hanousek Čiča *et al.*, 2024), or involved comparisons of various solvents and extraction methods (Stojiljković *et al.*, 2016). Ethanol has frequently been employed as a solvent in studies investigating bioactive compounds (Akinmoladun *et al.*, 2022, Liaudanskas *et al.*, 2014).

This study acknowledges several limitations. A

primary constraint is the choice of apple variety and its source. The chemical composition of apples is influenced by genetic factors and growing conditions, which introduces variability. Future studies should investigate the same apple variety under different environmental conditions (e.g., across growing seasons) and extend the analysis to other apple varieties used in the maceration process. Such approaches would provide a more comprehensive understanding of the factors affecting the health-promoting properties of alcoholic apple macerates.

### Conclusion

The study highlights the critical influence of ethanol concentration on the extraction efficiency of bioactive and nutritional components from apples. Among the tested macerates, the 40% ethanol extract emerged as the most effective in retaining polyphenols, achieving significantly higher levels compared to 20% and 70% ethanol concentrations. This finding underscores the pivotal role of ethanol concentration in optimizing polyphenol extraction. Vitamin C content was the highest in the 20% ethanol macerate, indicating that lower ethanol concentrations are more favorable for preserving this nutrient. However, the reduction in vitamin C content across all ethanol concentrations suggests that ethanol-based extraction may inherently limit its retention. Sugar content was also influenced by ethanol concentration, with the 40% ethanol macerate showing the highest sugar levels, closely followed by the 20% ethanol macerate. The 70% ethanol macerate demonstrated the least efficacy in preserving sugars, aligning with the observed trends for polyphenols and vitamin C. Overall, the study provides insights into the effects of ethanol concentration on the extraction of key nutritional and bioactive compounds from apples. Future research should explore the interplay between apple variety, growing conditions, and ethanol-based extraction to refine these findings and expand their applicability.

### Authors' contributions

K Sikorska-Zimny designed, conducted the

research, analyzed data, performed statistical analysis, wrote the paper, had primary responsibility for final content; E Bialek, A Kocik, M Koziol, and M Ziarkowska conducted the research; W Mielicki, M Wojciechowska provided essential reagents; and M Ziarkowska provided essential materials. All authors read and approved the final manuscript.

### Conflicts of interest

The authors declared no conflicts of interest.

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