

IMPACTS OF THE USE OF GLUTEN-FREE CEREALS AND SPICES ON THE QUALITY PARAMETERS OF BEER

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ABSTRACT

The focus of this study was to examine the brewing performance quality attributes of wort and beer produced entirely from such combinations at various quantities. The physiochemical characteristics of the different gluten-free beer with and without the addition of ginger in comparison to barley-based beers was also examined. In this study, three different types of gluten-free cereals (oat, rice and corn) were used in the production of pale beer. Freshly grated ginger was added to some of the produced beer samples prior to maturation. This was compared to the beer samples produced using barley malt and also with an addition of ginger. For this, the total phenolic content, flavonoid content, color intensity, bitter acid content, and selected mineral content have been evaluated.

The final product presented the bitter acid content of oat + rice beer with the highest amount among all the individual beer samples analyzed; In the Flavonoid content, the control sample beer was higher than all the other individual beer samples and the TPC showed a similar pattern. Concerning the mineral level present, potassium and phosphorus was seen to possess the highest concentration respectively in the beer samples. In addition, the oat beer has the highest amount in sodium, calcium and sulphur respectively while magnesium was highest in control + ginger beer.

The chemometric approach shows that the gluten free beer produced with and without ginger displayed several traits from the control samples. The cereal blends show some promising attributes as an adjunct for beer brewing.

Keywords: Gluten-free cereals, Beer, Ginger, Celiac disease

INTRODUCTION

Beer is a fermented and commonly consumed alcoholic beverage existing for a long period of time globally. Since wine and other beverages are typically more expensive than beer (Colen and Swinnen, 2015), the brewing industry has improved over time meeting the consumers changing demands (Ducruet *et al.*, 2017). There are various characteristics that are seen in beer. According to Koren *et al.* (2020) color of a beer is of great importance as it's the first attribute observed by the consumers. The European Brewery Convention (EBC) is one of the standard methods acceptable for determining the color in beer products, and another is the Standard Reference Method (SRM). When the EBC value is higher, the beer is described to be darker and pale when it is low. Iso-alpha-acids give the typical bitter taste to beer, which is the most important fraction of hops. Although complexation with residual sugars also contributes to the taste perceived by the consumers (Keukeleire, 2000). Phenolic compounds in beer originate from either cereal or hops and play critical roles in the color, flavor stability, colloidal stability and other properties of beer. Also, they are generally considered as one of very important antioxidant sources in beer (Vanderhaegen *et al.*, 2006). Phenolic content and antioxidant activity of beer depends on the quantity and quality of starting materials and on the brewing process itself (Callemien *et al.*, 2006). Flavonoid compounds are the largest group of polyphenolic substances. In beer, the type of cereal or hop used for brewing is of great importance as it contributes a large portion of the flavonoid concentration (Wannenmacher *et al.*, 2018).

Among the consumers of beer, a percentage of them are suffering from gluten intolerance. Due to this condition, food and beverages made from alternative gluten free cereals (millets, maize, sorghum, oat, rice) or pseudo cereals (quinoa, amaranth, buckwheat) are required in gluten-free diet (Hager *et al.*, 2012; Chiba *et al.*, 2012). The reduced amount of such gluten free products results in the consumers not having good access to steady health benefits and social sacrifices; often enduring the side effects such as stomach problems or diarrhea in order to take part in regular activities like hang-out or drinking beer at various occasion (Hager *et al.*, 2012).

Celiac disease (which is enteropathy related) is a prolonged systemic, autoimmune disorder in genetically susceptible persons, which is initiated by the consumption of dietary gluten from wheat, rye or barley, leading to the

inflammation of the small intestinal mucus membrane and villous atrophy. This condition is completely reversible once gluten is eliminated entirely from the diet (Nasr *et al.*, 2016). The consumption of gluten by celiac patients leads to several gastrointestinal and non-gastrointestinal disorders and biochemical irregularities which are resolved after the removal of gluten (Green and Jabri, 2003).

Oat (*Avena sativa* L.) has been an alternative gluten-free raw material in the food industry since it contains nearly no gluten making it suitable for gluten free food production (Arendt and Zannini, 2013). It has been of interest for individuals suffering from celiac disease. Research has shown that most celiac disease patients can tolerate oats. In addition, its nutritional profile is characterized by a relatively high amount of husk, β -glucan, protein and fat, and thus allow extract content (Schnitzenbaumer and Arendt, 2014). According to Munoz-Insa *et al.* (2011), oat has the potential for malting purposes, which makes it possible to produce oat malt that can be the basis for other sources of food, beverages, and beer production. Also, uses of oat for human consumption and nutrition in food has been expanded. Rice (*Oryza sativa* L.) has been existing for a long time as a common source of gluten free grain. It is considered as a non-expensive source of nutrient having more than three-quarter its percentage to be starch and its proteins are friendly to coeliac disease patients. In the production of beer, rice is mostly used as an adjunct (Hager *et al.*, 2014). Maize (*Zea mays*) is grown around the world and has the highest production quantity when compared with wheat and rice in the worlds grain statistics (FAO, 2020). It's also a common raw material used as an adjunct in breweries recently. Maize could be used as a cheap substitute for barley malt since it also gives the beer similar properties if used in a pregelatinized grits or flakes.

Ginger (*Zingiber officinale*) is majorly cultivated in the equatorial regions (Haniadka *et al.*, 2013). It is an important spice crop of both commercial and medicinal importance, widely used globally both in fresh and dried form which adds flavor by creating spicy pungent taste. Ginger contains polyphenol compounds that accounts for its unique aroma and therapeutic properties (Kaushal *et al.*, 2017).

The purpose of this study was to prepare different pale beers by using selected gluten-free raw materials that will be appropriate for people with gluten intolerance. The brewing performance quality attributes of wort and beer produced entirely from malted oat grains alone, malted oat with corn flakes and also with rice flakes mixed with various quantities was examined. Also, the

physiochemical characteristics of the different gluten-free beer with the addition of ginger in comparison to barley-based beers, as well as the beer having ginger was examined among others.

MATERIAL AND METHODS

Brewing technology

The production process of the gluten-free beer was done in January 2020 in the Institute of Food Science, University of Debrecen. All the raw ingredients used during the production process was bought from a brewing specialty shop in Budapest, Hungary, which includes malted oat cereal grain, rice flake, corn flake, dried brewer's ale yeast (*Saccharomyces cerevisiae*), hop pellets and malted barley cereal grains which was used for the production of the control sample. The fresh ginger was gotten from a mall in Debrecen, Hungary. Prior to the brewing process, the malted grains were kept at room temperature in the laboratory, while the hop was kept in a refrigerator to avoid the loss of volatile compounds.

The weighed malt (4kg of malted barley/ malted oat only; 2kg malted oat and 1.5kg corn/ rice flakes) alongside some volume of water (15 L) was put into the mash kettle and heated. For the digestion of proteins and starch to be performed, the mash was allowed to rest on three exact temperatures (52 °C for 15 min, 60 °C for 20 min, and 67 °C for 50 min) which are optimal for the required enzymatic reactions. By further heating to 80 °C, enzymes were inactivated, and the wort was then subjected to the iodine test. The same increasing temperature mashing process was used for the other malt blends.

Afterwards, the wort was taken out from the mash kettle, and the remaining extract was leached from the grains. About 7 L of water at 80 °C was used for the lautering process. After removing the spent grains, the wort was poured back into the tank and was heated until boiling point. Hop pellets (30g) were added to it right after reaching the boiling point to give bitterness to the beer and the timer was set for half an hour. Afterwards, another hop pellets of 20g were added into the tank for 50 minutes of boiling. At the end, the heating and circulation was stopped. The wort was cooled down and it was transfused into the fermentation tank by using an aseptic filter. For the fermentation process, the wort was inoculated by using dried yeast. The duration of the fermentation was 10 days at 18°C.

Prior to the maturation process (three weeks), priming sugar solution which was previously prepared was added to each bottle. Also, 2.65 g of the fresh ginger paste was added to some bottles containing the sugar solution. The quantity of ginger incorporated was established based on the study of **Tozetto et al. (2019)**. The bottles were then filled with the beer and the crown cork was placed on top of the bottle for closure. It was then stored in a cool, dark place.

Physicochemical analysis

Firstly, the samples were degassed by using an ultrasonic water bath (Bandelin Sonorex Digital DT 255H, Germany) and filtered through folded filter paper. The analysis carried out on the produced beverages includes total phenolic content, flavonoid content, color intensity, bitter acid content, vitamin c content and mineral content. The analytical measurements were carried out in triplicate.

Total phenolic content was determined according to the method described by **Singleton et al. (1999)**. Principle of the method is based on the property of Folin-Ciocalteu reagent that it oxidizes phenolic compounds due to the phosphotungstic acid and phosphomolybdic acid it contains. As a result, the color of the solution turns into blue in proportion to the quantity of phenolic compounds. Absorbance of the sample is measured with spectrophotometer (Evolution 300 LC, Thermo Electron Corporation, England) at a wavelength of 760 nm, against the mixture of methanol and distilled water (80:20). Applied chemicals: 3,4,5-trihydroxybenzoic acid (Alfa Aesar GmbH & Co. KG, Karlsruhe, Germany), sodium carbonate, methanol (Scharlab S.L., Spain) and Folin-Ciocalteu reagent (VWR International S.A.S., France). Results are expressed in mg GAE/ 100 ml (mg gallic acid equivalent/100 ml).

Flavonoid content was determined according to the method described by **Kim et al. (2003)**. After the addition of the chemicals, a rose-color complex is produced, which is measured by a spectrophotometer (Evolution 300 LC, Thermo Electron Corporation, England) at a wavelength of 510 nm, against blank solution. Applied chemicals: Catechin (Cayman Chemical Company, USA), aluminium chloride (Scharlab S.L., Spain), sodium nitrite (Scharlab Chemie S.A., Spain), sodium hydroxide (Sigma-Aldrich Chemie GmbH, Germany) and methanol (Scharlab S.L., Spain). Results are expressed in mg CE/100 ml (mg catechin equivalent/100 ml)

In case of color intensity, the degassed beer sample was poured into a cuvette and absorbance (A) was measured with a spectrophotometer (Evolution 300 LC, Thermo Electron Corporation, England) at 430 nm against distilled water. For the calculated result the equation below was used: Color intensity = $10 \times A_{430}$. By using this equation, color intensity value is calculated on the SRM (Standard Reference Method) scale. EBC (European Brewing Convention) scale is more common in the brewing industry. Use the following formula to calculate the results in EBC: EBC = SRM $\times 1.97$ (**Czipa, 2014**).

The bitterness was examined by the extraction of the substances behind the hops' bitterness, the alpha and beta acids with isooctane (2,2,4-trimethylpentane) from a medium acidified with hydrochloric acid and measure the quantity of α -bitter acids with a spectrophotometric method **Philpott et al. (1997)**. Applied chemicals: 2,2,4-trimethylpentane (VWR International S.A.S., France), hydrochloric acid (Sigma-Aldrich Chemie GmbH, Germany). Absorbance was measured at 275 nm against isooctane, results are expressed in mg/l.

To measure element content, a sample preparation method described by **Kovács et al. (1996)** was performed. To 20 ml of the degassed samples, 10 ml of concentrated HNO₃ (69% V/V, VWR International Ltd., Radnor, USA) was added, then samples were allowed to rest overnight. Then, samples were pre-digested at 60 °C for 30 min. After cooling, 3 ml of H₂O₂ (30% V/V, VWR International Ltd., Radnor, USA) was added, and the samples were digested at 120 °C for 90 min. Finally, samples were diluted to 50 ml by using ultrapure water (Milli-Q water purification system; Millipore SAS, Molsheim, France) and filtered through qualitative filter paper (grade: 388; Sartorius Stedim, Biotech S.A., Gottingen, Germany). Element contents were determined by using ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry) (Thermo Scientific iCAP 6300, Cambridge, UK).

The analysis was carried out for the samples seen in Table 1.

Table 1 Samples produced for the analysis

Sample No.	Sample type	Sample No.	Sample type
1	Control sample	5	Oat + Corn
2	Control + ginger	6	Oat + corn + ginger
3	Oat only	7	Oat + rice
4	Oat + ginger	8	Oat + rice + ginger

Source: Own editing

RESULTS AND DISCUSSION

Total phenolic, flavonoid, color intensity and bitter acid content of the beer samples

Total phenolic content, flavonoid content, color intensity and bitter acid content of the samples are presented in Table 2. From the Table, the TPC concentration follows the order 2>1>4>3>5>7>6>8. The lowest TPC concentration was obtained in sample 8 (20.7 mg GAE/100ml) while the highest TPC concentration was recorded in sample 2 (51.0 mg GAE/100ml). In addition, there was an overall increase in TPC results of sample 2 (51.0 mg GAE/100ml) and sample 4 (34 mg GAE/100ml) and decrease in sample 6 (22.6 mg GAE/100ml) and sample 8 (20.7 mg GAE/100ml) due to the addition of ginger. From the study, it was deduced that the use of oat malt in brewing of the beer had an obvious negative effect on the phenolic content. This was evident in the low concentration from samples 3 (30 mg GAE/100ml) to 8 (20.7 mg GAE/100ml) which were mixed with corn and rice flakes when compared with the control sample (barley) beer. The extract content of individual beer samples was not measured. This trend could not be changed with the addition of corn and rice either. The addition of ginger had different effects on different beer samples, but these effects were not significant compared to the effect of the raw materials. In addition, having beer samples that are rich in phenolic antioxidants indicates higher quality, more stable sensory properties, such as color, flavor and aroma, foam stability, and longer shelf life when compared to beer with lower antioxidant activity (**Woffenden et al., 2001**).

The flavonoid content of the beer samples had wide range of values. The control sample had the highest concentration (6.79), while sample 8 had the lowest concentration (2.09). From the Table, brewing with oat and the addition of both corn and rice flakes yielded no positive result as observed for TPC. The flavonoid concentration was far lower than the control sample (barley). The addition of ginger produced different results. In case of the control sample and the oat beer, similar results could be observed after the addition of ginger. But then again, the ginger increased the flavonoid content of oat + corn beer and decreased it in case of oat + rice beer. Besides these observations, low flavonoid content of these samples should be noted.

The produced beer samples showed low flavonoid content. Out of those, the highest flavonoid content could be observed in case of the control samples. The use of oat, corn and rice decreased flavonoid content in every case – no surprise, the source of these compounds is the raw material itself. The addition of ginger did not have noticeable effect in case of the control samples and the oat beer, but it did have some effect on the samples produced by using corn and rice.

The color intensity of the analyzed beer samples is also presented in Table 2. Every sample showed EBC values under 20. Sample 5 had the lowest EBC value, while sample 3 had the highest EBC value. This indicates that these beer samples are classified as pale beer according to the classification by **Codex Alimentarius Hungaricus (2013)**. The low values obtained in sample 5 and 6 could be associated with a decreased amount of nitrogen compounds being present for Maillard reactions from the corn flakes. Similar result was also obtained when

corn adjunct was added in different ratios to barley malt, which resulted in a reduction in the color of the wort (Poreda et al., 2014). Samples which contained only malted grains (barley or oat) showed higher color intensity than samples containing corn or rice flakes. Furthermore, the addition of ginger slightly decreased color intensity for all the samples except samples 5 and 6. In case of bitter acid content, the control samples (barley; barley + ginger) showed the highest bitter acid content except for sample 7. The result of the iso-alpha-acids which gives the typical bitter taste to beer is presented in Table 2. Sample 7 had the highest bitter acid content followed by the control samples 1 and 2. This could be related to the protein content and acidity of the raw materials. Increased pH and protein content may have a negative impact on the utilization of bitter acids (Bajkai et al., 2016). On the other hand, the addition of ginger did not have conspicuous effect on the bitter acid content in general and the use of oat malt decreased bitter acid content compared to the control sample. Although complexation with residual sugars also contributes to the taste perceived by the consumers (Keukeleire, 2000).

Table 2 Total phenolic content, flavonoid content, color intensity and bitter acid content of the samples

Sample	TPC (mg GAE/100 ml)	Flavonoid (mg CE/100 ml)	Color Intensity (EBC)	Bitter Acid (mg/l)
1	50.0 ± 1.0	6.79 ± 0.05	12.7 ± 0.3	83.7 ± 2.3
2	51.0 ± 1.0	6.27 ± 0.23	10.9 ± 0.2	83.8 ± 1.4
3	30.0 ± 0.7	3.84 ± 0.18	12.9 ± 0.2	80.8 ± 1.0
4	34.0 ± 1.5	3.75 ± 0.15	11.4 ± 0.1	81.9 ± 1.1
5	26.5 ± 0.7	2.79 ± 0.25	4.3 ± 0.10	78.0 ± 0.8
6	22.6 ± 0.0	4.38 ± 0.48	4.52 ± 0.06	78.0 ± 0.5
7	23.1 ± 1.6	3.13 ± 0.28	10.2 ± 0.13	87.7 ± 0.1
8	20.7 ± 0.6	2.09 ± 0.13	6.17 ± 0.24	79.7 ± 0.9

Source: Own editing

Selected mineral content level of the analyzed beer samples

The mineral content level of the analyzed beer samples in the study is presented in Table 3.

The raw ingredients used in beer brewing as well as industrial processing and containers, all contributes to the mineral presence seen in beers (Li et al., 1993). From the Table below, there was wide variations in concentration of potassium across the samples. The lowest concentration was recorded in sample 7 (249 mg/l) while the highest concentration was 564 mg/l recorded in both sample 1 and sample 2. The concentration of the other samples was below the control samples. There was significant decrease in the potassium level when oat malt was added, and slight decrease when rice and corn flakes were added. The addition of ginger did not have much effect to the potassium level of the individual beer samples without ginger.

The sodium range was not too wide. The highest concentration was seen in sample 3 (40.4 mg/l), oat beer, while both the control beer sample and control + ginger beer was observed to have the lowest concentration of 31.9 mg/l in the analyzed samples. From the results obtained, it is noticed that the other beer samples had higher sodium content level when compared to that of the control

beer. Also, the addition of ginger to all the beer samples did not have much effect to the sodium levels seen in the beer samples. Except that of sample 7 (oat + rice beer) which was 33.3 mg/l and an increment was seen to 37.7 mg/l in sample 8 (oat + rice + ginger beer).

The highest concentration of calcium level was seen in sample 3 oat beer (58.4 mg/l), followed by its ginger-enriched form (55.7 mg/l), while the calcium concentration with the lowest content was seen in sample 1 control beer (29.3 mg/l). The addition of both corn and rice (+ ginger) as seen in samples 5 to 8 respectively to the oat, did not have a positive result. As there was a reduction in the corresponding concentration levels compared to that of sample 3 (oat beer). As a result, oat beers prepared with the addition of corn and rice flakes had only a bit higher calcium content than the control sample, instead of the high concentration observed in pure oat beers. The use of oat malt instead of barley decreased mineral concentrations in most cases, except for Calcium and Sulphur. The addition of ginger did not have conspicuous effect on the mineral concentrations in general, only slight changes may be observed.

The observed range of magnesium was a bit wide. The highest concentration was seen in sample 2 control + ginger beer (63.6 mg/l), while the magnesium concentration with the lowest content was seen in sample 7 oat + rice beer (33.1 mg/l). The addition of ginger to all the beer samples respectively had a positive outcome. There was a bit increment in all the ginger beer samples compared with the samples without the ginger. In addition, there was a strong decrease in the beer samples containing both corn and rice respectively unlike the other beer samples.

The highest concentration of phosphorus was 256 mg/l seen in both samples 1 and 2, control beer and control + ginger beer, while the phosphorus concentration with the lowest content was seen in sample 7 oat + rice beer (69.9 mg/l). All the analyzed beer samples had phosphorus level far below the control sample beer. Also, the combination of both corn and rice respectively to the oat beer, had a negative outcome on the phosphorus level when compared to the oat beer only. The addition of ginger to the individual beer samples resulted in a little increment in the phosphorus concentration, except in that of the control beer which had the same as the control + ginger beer. From all the mineral elements analyzed in the beer samples, it was noticeable that potassium and phosphorus had the highest concentration. The same pattern was recorded according to Alcázar et al. (2002), when quantifying the mineral content of 32 samples of lager type beer from Spain and recorded the values for potassium and phosphorus to be the highest respectively. Also, Tozetto et al. (2019) observed potassium to be the highest concentration with 412.5 mg/l in the craft beer with ginger. According to Alcázar et al. (2002), the high concentrations may have originated in the raw materials (hops and type of cereals) used in the brewing process. Although, yeast is also a rich source of these elements.

Sulphur level was seen to be highest in sample 3 oat beer (109 mg/l), while the sulphur concentration with the lowest content was seen in sample 1 control beer having 49.7 mg/l (table 3). The addition of ginger did not have much effect on the sulphur levels of the individual beer samples either. Also, the brewing of corn and rice respectively with the malted oat grains caused a reduction in the sulphur content (samples 5 to 8) unlike oat only having a far higher concentration. Because of this, beers prepared by the addition of corn and rice had similar sulphur content to the control sample.

Table 3 Selected mineral content level of the analyzed beer samples.

Sample	K (mg/l)	Na (mg/l)	Ca (mg/l)	Mg (mg/l)	P (mg/l)	S (mg/l)
1	564 ± 3	31.9 ± 0.1	29.3 ± 1.5	61.3 ± 0.2	256 ± 1	49.7 ± 0.6
2	564 ± 2	31.9 ± 0.1	29.8 ± 0	63.6 ± 0.2	256 ± 1	51.1 ± 0.1
3	397 ± 22	40.4 ± 1.8	58.4 ± 4.2	57.1 ± 3.8	153 ± 4	109 ± 8
4	393 ± 7	40 ± 0.7	55.7 ± 1.5	57.6 ± 1	157 ± 4	105 ± 2
5	311 ± 19	36.6 ± 2.3	32.2 ± 1.5	35.9 ± 1.9	76.6 ± 4.7	52.1 ± 2.7
6	340 ± 8	38.9 ± 1.1	31.2 ± 0.9	39.7 ± 0.9	83.2 ± 1.7	54.9 ± 1.7
7	249 ± 14	33.3 ± 2.1	37.9 ± 0.8	33.1 ± 1	69.9 ± 7.4	51.3 ± 0.8
8	272 ± 3	37.7 ± 0.6	33.1 ± 0.3	34.2 ± 0.2	72.8 ± 1.3	50.4 ± 0.8

Source: Own editing

CONCLUSION

In this study, eight beer samples were produced and analyzed from different gluten-free cereals with the incorporation of ginger to some samples. The purpose was to prepare different pale beers by using oat, rice and corn as the main raw materials. The incorporation of ginger paste was done prior to the maturation stage. The produced beer samples were compared with the control beer sample which had barley malt as its major raw material. From the measured parameters, the use of oat instead of barley had a negative effect on the phenolic content, just like in case of flavonoids. This trend could not be changed with the addition of corn and rice either. All the analyzed beer samples had low EBC values which indicates that the produced were all pale beers. Using oat malt instead of barley decreased mineral concentrations in most cases, except for Calcium and Sulphur. Also, the addition of ginger did not have conspicuous effect on the mineral concentrations in general, only slight changes may be observed.

The preparation of beers by using gluten-free ingredients is extremely beneficial for people suffering from celiac disease. By consuming these beverages, they can also experience the joy of tasting unique alcoholic products. On the other hand, based on the results, the use of alternative cereals has negative effect on the nutritional parameters of beer in most cases. Of course, the primary objective of beer consumption is not its potential health benefit, more likely its enjoyment value.

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