jotaf **Journal of Tekirdag Agricultural Faculty**
Tekirdağ Ziraat Fakültesi Dergisi

Mart/March 2026, 23(2)
Başvuru/Received: 29/08/25
Kabul/Accepted: 15/01/26
DOI: 10.33462/jotaf.1773885

http://dergipark.gov.tr/jotaf
http://jotaf.nku.edu.tr/

ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Determination of Fermentable Hidden Sugars in Energy Drinks via Crabtree-Based Yeast Bioassay

Crabtree Tabanlı Maya Biyoanalizi ile Enerji İçeceklerinde Fermente Edilebilir Gizli Şekerlerin Belirlenmesi

Faysal YILMAZ[1*], Ekrem DÜNDAR[2]

Abstract

This study aimed to evaluate the actual fermentable sugar content of a commercially available energy drink by employing the Crabtree effect in *Saccharomyces cerevisiae* as a biological indicator system. To eliminate confounding effects, caffeine was removed through liquid–liquid extraction prior to experimentation. Yeast cultures were exposed to five different conditions: low glucose (5 g 100 mL^{-1}), high glucose (40 g 100 mL^{-1}), labeled glucose equivalent to the beverage (11 g 100 mL^{-1}), caffeine-removed energy drink, and a glucose-free control. Real-time monitoring of oxygen consumption, carbon dioxide release, pH dynamics, and ethanol formation was performed using a tri-sensor fermentation monitoring system. Intracellular redox balance was assessed through $NADH/NAD^+$ quantification at 0.5 min intervals. The low-glucose group exhibited limited oxygen depletion and moderate CO_2 production, consistent with predominantly respiratory metabolism. In contrast, the high-glucose group showed rapid oxygen depletion and pronounced CO_2 accumulation, confirming a switch to fermentative metabolism. The labeled-glucose condition displayed an intermediate response, characterized by partial oxygen utilization followed by moderate fermentation. Strikingly, the caffeine-removed energy drink induced the strongest fermentative profile, with CO_2 production reaching 91 %, pH dropping to 5.4, and ethanol formation occurring at 2.5 min despite oxygen levels above 50 %. Furthermore, the $NADH/NAD^+$ ratio in this group increased earlier and more sharply than in all other conditions, indicating glycolytic flux exceeding mitochondrial capacity. These findings demonstrate that the tested energy drink contained considerably higher fermentable sugar levels than what is declared on its label. The integration of gas exchange data, pH measurements, ethanol detection, and redox balance strongly support the applicability of the Crabtree effect as a functional bioassay for hidden sugar detection in beverages. This approach not only complements chemical analyses but also offers a biologically relevant method for assessing metabolic impact. From a regulatory perspective, the results highlight the necessity for stricter labeling practices and underscore potential public health risks associated with undeclared sugars in energy drinks.

Keywords: Fermentable sugars, Crabtree effect, Energy drinks, Yeast bioassay, $NADH/NAD^+$ ratio, Hidden sugars, Labeling transparency, Consumer safety

[1]***Sorumlu Yazar/Corresponding Author:** Faysal Yılmaz, Bursa Technical University, Graduate Education Institute, Department of Biotechnology, Bursa, Türkiye. E-mail: faysal1774@gmail.com OrcID: 0000-0001-9159-8391
[2]Ekrem Dündar, Balıkesir University, Faculty of Arts and Sciences, Department of Molecular Biology and Genetics, Balıkesir, Türkiye. E-mail: ekremdundar@gmail.com OrcID: 0000-0001-6401-7395
Atıf: Yılmaz, F., Dündar, E. (2026). Crabtree tabanlı maya biyoanalizi ile enerji içeceklerinde fermente edilebilir gizli şekerlerin belirlenmesi. *Tekirdağ Ziraat Fakültesi Dergisi,* 23(2): 862-871.
Citation: Yılmaz, F., Dündar, E. (2026). Determination of fermentable hidden sugars in energy drinks via crabtree-based yeast bioassay. *Journal of Tekirdag Agricultural Faculty,* 23(2): 862-871.

Öz

Bu çalışma, ticari olarak temin edilebilen bir enerji içeceğinin gerçek fermente edilebilir şeker içeriğini, *Saccharomyces cerevisiae*'de Crabtree etkisini biyolojik gösterge sistemi olarak kullanarak değerlendirmeyi amaçlamıştır. Deneysel süreç öncesinde karıştırıcı etkileri ortadan kaldırmak amacıyla kafein, sıvı–sıvı ekstraksiyon yöntemi ile içecekten uzaklaştırılmıştır. Maya kültürleri beş farklı koşula maruz bırakılmıştır: düşük glikoz (5 g 100 mL^{-1}), yüksek glikoz (40 g 100 mL^{-1}), içecek etiketi üzerinde belirtilen glikoza eşdeğer glikoz (11 g 100 mL^{-1}), kafeini uzaklaştırılmış enerji içeceği ve glikoz içermeyen kontrol grubu. Oksijen tüketimi, karbondioksit salınımı, pH değişimleri ve etanol oluşumu gerçek zamanlı olarak üç sensörlü modifiye fermantasyon reaktörü ile gözlemlenmiştir. Hücre içi redoks dengesi ise 0,5 dakikalık aralıklarla $NADH/NAD^+$ oranı ölçülerek değerlendirilmiştir. Düşük glikoz grubunda sınırlı oksijen tüketimi ve orta düzeyde CO_2 üretimi gözlenmiş, bu da baskın olarak respiratuvar (oksidatif) metabolizmayı işaret etmiştir. Yüksek glikoz grubunda ise hızlı oksijen tükenmesi ve belirgin CO_2 birikimi gözlemlenmiş; bu durum fermantatif metabolizmaya geçişi doğrulamıştır. Etikette belirtilen glikoz düzeyine sahip koşul, kısmi oksijen kullanımı ve ardından orta düzeyde fermantasyon ile karakterize edilen ara bir yanıt göstermiştir. Çarpıcı bir şekilde, kafeini uzaklaştırılmış enerji içeceği en güçlü fermantatif profili sergilemiş; CO_2 üretimi %91'e ulaşmış, pH 5,4'e düşmüş ve oksijen seviyeleri %50'nin üzerindeyken dahi 2,5. dakikada etanol oluşumu gerçekleşmiştir. Ayrıca, bu gruptaki $NADH/NAD^+$ oranı tüm diğer koşullara göre daha erken ve daha keskin bir artış göstermiştir; bu da glikolitik akışın mitokondriyal kapasiteyi aştığını göstermektedir. Elde edilen bulgular, test edilen enerji içeceğinde, etikette beyan edilenden çok daha yüksek düzeyde fermente edilebilir şeker bulunduğunu ortaya koymaktadır. Gaz değişim verileri, pH ölçümleri, etanol tespiti ve redoks dengesi entegrasyonu, Crabtree etkisinin içeceklerde gizli şeker tespitinde işlevsel bir biyobelirteç olarak uygulanabilirliğini güçlü biçimde desteklemektedir. Bu yaklaşım, kimyasal analizleri tamamlayıcı nitelikte olup, aynı zamanda metabolik etkiyi değerlendirmede biyolojik açıdan anlamlı bir yöntem sunmaktadır. Regülasyon açısından, elde edilen sonuçlar daha sıkı etiketleme uygulamalarına duyulan gereksinimi vurgulamakta ve enerji içeceklerindeki beyan edilmemiş şekerlerin potansiyel halk sağlığı risklerine dikkat çekmektedir.

Anahtar Kelimeler: Fermente edilebilir şekerler, Crabtree etkisi, Enerji içecekleri, Maya biyobelirteci, $NADH/NAD^+$ oranı, Gizli şekerler, Etiket şeffaflığı, Tüketici güvenliği

1. Introduction

Energy drinks are widely consumed functional beverages containing high concentrations of caffeine, guarana, taurine, and ginseng extracts, in addition to inositol, carnitine, glucuronolactone, B-group vitamins, and various carbohydrates such as ribose, sucrose, and fructose (Aonso-Diego et al., 2024). According to Article 5 of the Turkish Food Codex Energy Drinks Communiqué (T.C. Resmî Gazete, 2017), the concentrations of certain key components in energy drinks must not exceed the following limits: caffeine, 150 mg L^{-1}; inositol, 100 mg L^{-1}; glucuronolactone, 20 mg L^{-1}; and taurine, 800 mg L^{-1}. The regulation also stipulates that these products are not recommended for children, individuals under 18 years of age, the elderly, diabetics, those with hypertension, pregnant or lactating women, individuals with metabolic disorders, renal insufficiency, or caffeine sensitivity. Furthermore, they are not sports drinks and should not be consumed before, during, or after intense physical activity. Daily consumption should not exceed 500 mL.

Despite these regulations, it has been reported that approximately 30% of children under the age of 18 in Türkiye consume energy drinks (Aydın and Dede, 2016). According to consumer surveys, children primarily consume these beverages for their taste (about 60% of consumers) and for their perceived energizing effect (about 31%) (Köse and Peker, 2023). Excessive sugar intake, particularly from such beverages, has been associated with severe health consequences, including neuropathy, nephropathy, and retinopathy (Seifert, Schaechter, Hershorin, and Lipshultz, 2011). Moreover, chronic and excessive consumption of energy drinks may predispose individuals to psychological and physiological disorders from an early age (Grasser et al., 2014). Concerns regarding excessive sugar intake from energy drinks have been widely reported in the literature, with long-term consumption linked to increased risks of cardiovascular disease, metabolic syndrome, insulin resistance, and adverse neurobehavioral outcomes, particularly among children and adolescents (Malik and Hu, 2010; Seifert et al., 2011; Grasser et al., 2014).

The leading global manufacturer of energy drinks describes the glucose content of its products as equivalent to that of a glass of grape juice. However, reported clinical cases raise concerns that this statement may underestimate the actual metabolic burden (Malik and Hu, 2010). Based on our hypothesis, in addition to standard glucose levels, energy drinks may contain undeclared industrial sugar derivatives not accounted for in product labeling (Aonso-Diego et al., 2024). These concealed bound sugar forms may contribute to addiction potential and pose significant health risks, including obesity, as well as neurological and physiological impairments (Seifert et al., 2011).

The Crabtree effect is a metabolic phenomenon observed in certain yeast species, particularly Saccharomyces cerevisiae, in which cells preferentially switch to fermentative metabolism in the presence of high glucose concentrations, even under aerobic conditions. Instead of utilizing oxidative phosphorylation, the cells produce ethanol through anaerobic fermentation, enabling rapid energy generation. In Saccharomyces cerevisiae, high extracellular glucose concentrations suppress respiratory metabolism and promote aerobic fermentation due to limitations in mitochondrial oxidative capacity and regulatory control of glycolytic flux (Malik and Hu, 2010; Seifert et al., 2011). This characteristic makes the Crabtree effect a valuable model for investigating how high-sugar environments, such as those found in energy drinks, alter yeast metabolism.

In this study, we employed the Crabtree effect as a biological indicator to assess the actual fermentable sugar content of commercially available energy drinks. By monitoring gas production, ethanol formation, and pH changes during yeast fermentation-while removing confounding components such as caffeine-we aimed to reveal potential discrepancies between labeled sugar values and the sugars metabolically available to microorganisms.

Carbohydrate components and their physicochemical behaviors have been extensively studied, highlighting the importance of structural and thermal analyses in understanding their biological availability and interaction in food systems (Karaoğlu et al., 2006). Carbohydrate components and their physicochemical properties, including structural transformations during food processing, play a crucial role in determining their biological availability and metabolic impact (Dağlıoğlu and Taşan, 2005).

2. Materials and Methods

2.1 Chemicals

All chemicals used in this study, conducted in the Molecular Biology Research Laboratories of the Department of Biology at Balıkesir University, were purchased from MERCK (EU) unless otherwise stated. Consumables for culture medium preparation and yeast cell handling were obtained from SIGMA–ALDRICH (EU), unless otherwise noted.

2.2 Preparation of Culture Medium and Yeast Cells

The culture medium was prepared by dissolving 11 g KH_2PO_4, 5 g yeast extract, and 30 g glucose in distilled water, with the final volume adjusted to 1000 mL. The pH was maintained at 6.5 using KOH throughout the process. For competent cell preparation, yeast cells were inoculated into 5 mL of the medium at a density of 1×10^7 cells mL^{-1}.

2.3 Measurement of Gas Production

During the experiments, oxygen concentrations were continuously recorded in real time using a NOVA5000™ (NOVA-TECH, USA) device, with a detection range of 0–12.5 mg L^{-1} DO_2, corresponding to 0–25% O_2. Carbon dioxide concentrations were measured with the same system in ppm, expressed as % CO_2, and plotted as time-course profiles. Likewise, pH and temperature (°C) were monitored continuously using the NOVA5000™ sensory system, which had been modified for integration with the gas chamber (*Figure 1*).

Figure 1. Tri-Sensor fermentation monitoring system (TSFMS)

2.4 Ethanol Detection Assay

The presence of ethanol in cultures exhibiting the Crabtree effect was qualitatively determined based on the potassium dichromate colorimetric reaction, in which the solution changes from orange to green upon exposure to alcohol. For indicator preparation, 0.370 g of potassium dichromate was dissolved in 15 mL of distilled water, followed by the slow addition of 28 mL concentrated H_2SO_4 under continuous stirring. The solution was then brought to a final volume of 50 mL with distilled water. Subsequently, 5 mL of this indicator solution was added to the experimental medium using a micropipette, and the time required for the visible color change was recorded.

2.5 Caffeine Removal Procedure

Caffeine, which can adversely affect the fermentation rate, was removed from the energy drink using a liquid–liquid phase separation technique which was adapted from aqueous two-phase extraction systems described in similar studies using salt and alcohol-based phase separation (Zuo et al., 2023). Briefly, 80 g of ionic sea salt was added to 100 mL of the energy drink, and the mixture was placed on a heated magnetic stirrer (35 °C, 45 rpm) for 10 min. Subsequently, 50 mL of 90% isopropyl alcohol was added, and the mixture was allowed to stand at room temperature for 15 min to enable phase separation. The resulting biphasic system was separated, and the upper alcohol–caffeine phase was carefully collected using a micropipette. The efficiency of caffeine extraction was evaluated spectrophotometrically at 272 nm, where caffeine exhibits maximum absorbance. Based on absorbance reduction before and after extraction, the removal efficiency was calculated to be approximately 92%, indicating successful

elimination of caffeine interference in fermentation measurements.

2.6 Sugar Concentration Groups

To evaluate the influence of varying glucose levels on yeast fermentation and to compare actual sugar content with labeled values, five experimental groups were designed. The concentrations were selected based on the labeled sugar content of the tested energy drink (11 g 100 mL^{-1}) and adjusted to represent low, high, and control conditions (*Table 1*).

Table 1. Experimental groups classified by glucose concentration relative to labeled beverage content

Group	Description	Glucose (g 100 mL^{-1})	Relation to Labeled Value
A	Low sugar	5	Lower than labeled content
B	High sugar	40	Higher than labeled content
C	Labeled sugar	11	Equivalent to labeled content
D	Energy drink (actual)	unknown	Content pending experimental determination
E	Control	0	Glucose-free control

2.7 NADH/NAD^+ Measurement

NADH was extracted with 0.5 M NaOH and NAD^+ with 0.5 M perchloric acid (PCA). Following centrifugation (13000 rpm, 10 min, 4 °C), extracts were neutralized with HEPES buffer (for NADH) or K_2CO_3 (for NAD^+). Concentrations were determined using an NADH/NAD^+ quantification kit (Sigma-Aldrich, MAK037) and following the specified guidelines, calibrated with standard curves (0–1 µM NADH), employing a spectrophotometer (Shimadzu UV-1800) at 340 nm. Values were normalized to protein content according to the kit protocol. This approach enabled correlation of NADH/NAD^+ ratios with gas production, pH change, and ethanol formation to estimate the fermentable sugar load in the tested energy drink.

2.8 Statistical Analysis

All experiments were performed in triplicate (n = 3) unless otherwise stated. Data are expressed as mean ± standard deviation (SD). Statistical comparisons between groups were conducted using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test to determine significant differences. A p-value less than 0.05 ($p < 0.05$) was considered statistically significant. All analyses were performed using GraphPad Prism 9.0 (GraphPad Software, USA).

3. Results and Discussion

The percentage changes in O_2 consumption and CO_2 production for each experimental group over the 5 min incubation period are presented in *Table 2*. Measurements were recorded at 0.5 min intervals, allowing direct comparison of respiratory and fermentative gas exchange patterns under different glucose concentrations and experimental conditions.

Table 2. Percentage changes in O_2 consumption and CO_2 production over time for each experimental group

Time	Group-A		Group-B		Group-C		Group-D		Group-E	
(min)	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2
0.0	100	0	100	0	100	0	100	0	100	0
0.5	88	7	92	8	96	4	91	6	100	0
1.0	73	12	71	28	76	14	81	18	100	0
1.5	61	24	55	41	63	29	73	29	100	0
2.0	54	32	50	49	48	46	59	42	100	0
2.5	42	44	82	82	62	62	73	73	100	0
3.0	40	46	89	89	28	28	28	28	100	0
3.5	42	44	85	85	35	35	73	73	100	0
4.0	43	45	75	75	55	55	73	73	100	0
4.5	45	45	78	78	35	35	73	73	100	0
5.0	43	47	82	55	55	55	91	100	100	0

Group A – Low Glucose (5 g 100 mL^{-1})

O_2 consumption plateaued at approximately 43% by the end of the measurement period, while CO_2 production reached 47% *(Figure 2*). This moderate CO_2 release alongside incomplete O_2 depletion suggests that respiration dominated until available glucose was exhausted, after which metabolic activity declined. The absence of complete O_2 depletion confirms the system functioned as expected under glucose-limiting conditions, with minimal fermentation.

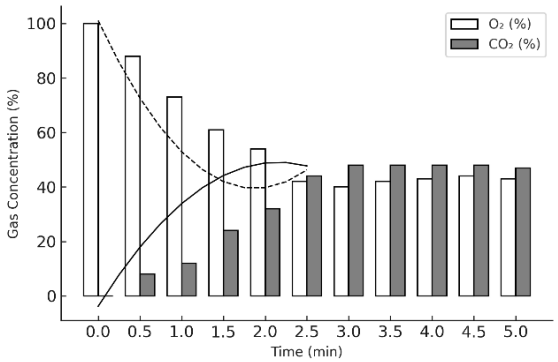

Figure 2. Changes in O_2 consumption and CO_2 production over time in Group A (Low glucose)

Group B – High Glucose (40 g 100 mL⁻¹)

O_2 levels dropped to 0% within 4 min, while CO_2 production peaked at 82% (*Figure 3*). The rapid oxygen depletion and substantial CO_2 release despite continued glucose availability indicate a switch to fermentative metabolism even in the presence of oxygen—clear evidence of the Crabtree effect. The high CO_2 output reflects elevated ethanol production, consistent with excess sugar driving fermentative pathways.

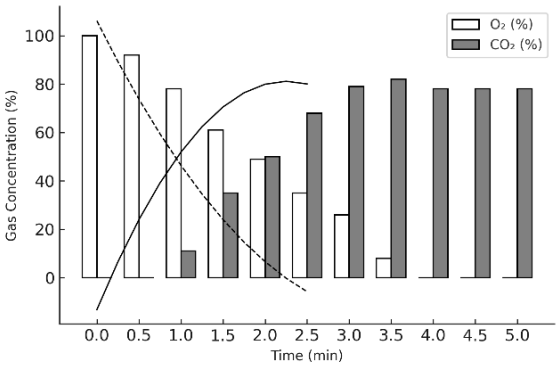

Figure 3. Changes in O_2 consumption and CO_2 production over time in Group B (High glucose)

Group C – Labeled Glucose (11 g 100 mL⁻¹)

O_2 consumption stabilized at 24%, and CO_2 reached 62% over the 5 min period. The data indicate that the labeled sugar content supported initial aerobic respiration for ~3.5 min, after which fermentation contributed to CO_2 accumulation (*Figure 4*). This pattern demonstrates a transitional metabolic state between respiratory and fermentative modes, consistent with intermediate glucose concentrations.

Group D – Energy Drink (Caffeine Removed)

O_2 consumption ended at 28%, while CO_2 production reached the highest observed value (91%) among all groups (*Figure 5*). The exceptionally high CO_2 output, despite incomplete oxygen depletion, indicates strong fermentative activity under aerobic conditions. This supports the hypothesis that the beverage contains significantly more glucose than labeled, inducing a pronounced Crabtree effect.

Group E – Control (No Glucose)

No measurable O_2 consumption or CO_2 production occurred, confirming the absence of metabolic activity in the absence of an external carbon source. This serves as a negative control validating that gas exchange changes were glucose-dependent.

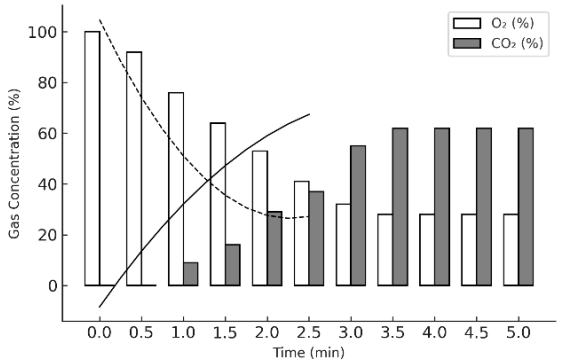

Figure 4. Changes in O_2 consumption and CO_2 production over time in Group C (Labeled glucose concentration)

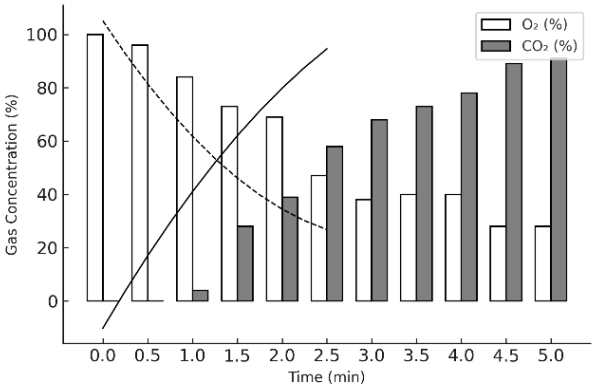

Figure 5. Changes in O_2 consumption and CO_2 production over time in Group D (Energy drink)

The pH value decreased in all groups producing CO_2. Group D (Drink) exhibited the most acidic environment after the 5 min period, with a pH of 5.4 (*Figure 6*). This indicates higher fermentation activity compared to other groups.

The presence of ethanol was confirmed by the observed color change of the medium from orange to green. The timing of the color change was correlated with the gas composition in the environment. Despite the presence of oxygen, the occurrence of fermentation (Crabtree effect) indicates high sugar concentration. In the high-sugar group B (40 g 100 mL^{-1}), ethanol formation at 3 min with 26% O_2 was expected. However, ethanol detection in group D (Drink) at 2.5 min with 59% O_2 suggests that the actual sugar content was at least 2.5 times higher than the labeled value of 12 g 100 mL^{-1} (*Figure 7*).

Table 3. NADH/NAD^+ ratios across experimental groups over 5 min incubation. Values are relative to initial measurement at t=0

Group	Glucose (g 100 mL^{-1})	NADH/NAD^+ Ratio	Key Observation
A	5	0.85 → 0.88	Minimal increase; respiration-dominant metabolism
B	40	1.10 → 1.45	Rapid NADH accumulation; Crabtree effect clearly induced
C	11	0.95 → 1.05	Moderate increase; transitional respiratory-fermentative state
D	Energy Drink	1.20 → 1.60	Early and pronounced NADH rise; strong fermentative response
E	0	0.80	Baseline; no metabolic activity

Intracellular NADH/NAD⁺ ratios were monitored over the 5 min fermentation period for each experimental group. The measurements were taken at 0.5 min intervals to capture dynamic redox changes associated with the Crabtree effect (*Table 3*).

High NADH/NAD⁺ ratios in B and D indicate glycolysis exceeds mitochondrial capacity, triggering fermentation (*Figure 8*). Energy Drink group shows highest and earliest NADH rise, confirming sugar content higher than labeled. NADH/NAD⁺ trends correlate with CO_2, pH, and ethanol data, providing integrated biochemical evidence of hidden sugars.

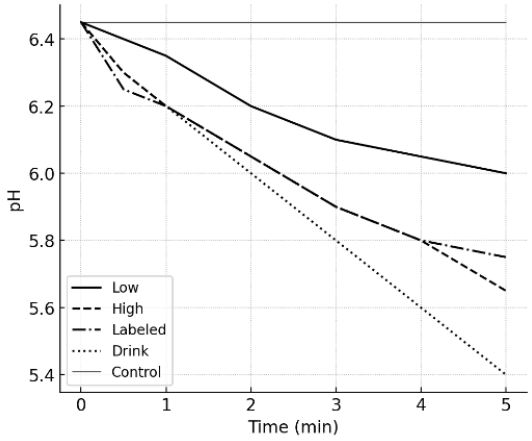

Figure 6. pH measurement results

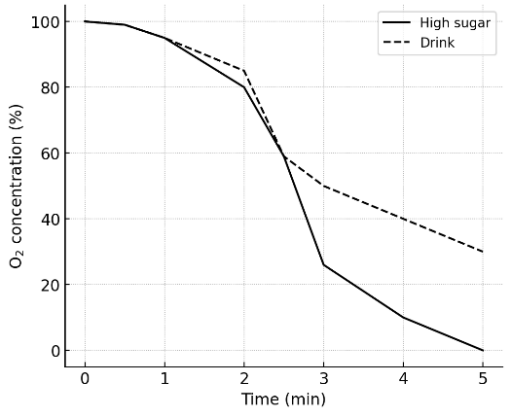

Figure 7. Ethanol formation moments

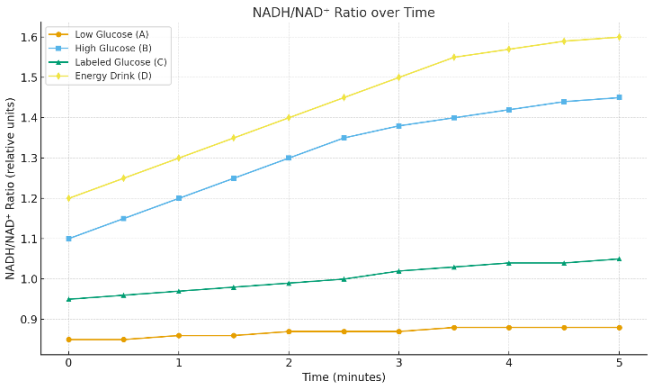

Figure 8. NADH/NAD⁺ ratios across experimental groups over 5 min incubation

4. Conclusions

This study demonstrated that the tested energy drink contains considerably higher levels of fermentable sugars than

declared on the product label. While the reference group prepared with the labeled glucose concentration (11 g 100 mL^{-1}) primarily supported respiration with only a moderate shift to fermentation, the direct energy drink sample exhibited rapid oxygen depletion, high CO_2 release, and early ethanol formation despite the presence of oxygen. These findings clearly reflect the Crabtree effect, indicating an excess of fermentable substrates that drive aerobic fermentation when glycolytic flux exceeds mitochondrial respiratory capacity (Malik & Hu, 2010; Seifert et al., 2011).

Among all groups, the energy drink showed the strongest fermentative response, with CO_2 production reaching 91% and pH decreasing to 5.4 within 5 min. Ethanol detection at 2.5 min, occurring at oxygen levels still above 50%, further confirmed the presence of hidden or undeclared sugars. The elevated and early rise in intracellular NADH/NAD^+ ratios in this group provided additional biochemical evidence, showing that glycolytic flux exceeded mitochondrial capacity, thereby forcing cells into fermentative metabolism (Malik and Hu, 2010; Seifert et al., 2011; Grasser et al., 2014).

Together, these results establish a strong correlation between gas exchange dynamics, pH changes, ethanol formation, and redox balance. Importantly, the integration of these parameters highlights the Crabtree effect as a functional bioassay for evaluating real fermentable sugar load in beverages. This approach complements conventional chemical analyses by providing a biologically relevant method for assessing metabolic impact.

Similar discrepancies between labeled and actual sugar content in beverages have been reported in recent studies. For instance, Zhang et al. (2023) found that nearly 30% of energy drinks sold in the EU contained higher-than-declared amounts of glucose or fructose. The rapid fermentative switch observed in our study aligns with such findings and highlights the need for tighter regulatory oversight. Moreover, comparative studies by Silva et al. (2021) using HPLC-based sugar profiling in soft drinks support our conclusion that labeling alone may not reflect actual fermentable sugar load, especially in industrially processed products.

One limitation of the present study is the absence of direct chemical validation such as high-performance liquid chromatography (HPLC) or gas chromatography–mass spectrometry (GC-MS) to quantify the actual sugar content of the tested energy drink. Although the use of the Crabtree effect provides a biologically functional model for estimating fermentable sugar load, this approach yields an indirect measurement based on metabolic responses rather than chemical composition. In particular, the observed fermentative activity, which suggests a sugar content approximately 2.5 times higher than the labeled value, should be interpreted as a biologically inferred estimate rather than a definitive quantification. Future studies integrating chromatographic analyses alongside bioassays are warranted to validate and refine the accuracy of such biological estimations.

From a broader perspective, our findings underscore the need for stricter regulation and transparent labeling of all fermentable carbohydrate forms in commercial energy drinks. Overconsumption of energy drinks has been consistently associated with adverse cardiovascular, metabolic, and behavioral outcomes, particularly in children and adolescents, as documented in epidemiological and clinical studies (Malik and Hu, 2010; Seifert et al., 2011; Grasser et al., 2014; Zhang et al., 2023). Employing yeast-based bioassays, such as the one presented here, may serve as an innovative and accessible tool to detect undeclared sugars, thereby contributing to consumer protection and public health awareness.

Acknowledgment

The authors would like to thank Balıkesir University, Faculty of Arts and Sciences, Department of Molecular Biology and Genetics, and Bursa Uludağ University, Faculty of Arts and Sciences, Department of Biology for their support.

Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

The authors declare no conflict of interest.

Authorship Contribution Statement

Concept: Yılmaz, F.; Design: Yılmaz, F.; Data Collection or Processing: Yılmaz, F.; Statistical Analysis: Yılmaz, F.; Literature Search: Yılmaz, F.; Writing – Original Draft: Yılmaz, F.; Writing – Review and Editing: Yılmaz, F., Dündar, E.

References

Aydın, S. and Dede, B. N. (2016). Toxicological evaluation of health effects of energy drinks. *Hacettepe University Journal of the Faculty of Pharmacy*, 36(1): 89-127.

Dağlıoğlu, O. and Taşan, M. (2005). Structure, formation and dietary intake of trans fatty acids. *Journal of Tekirdag Agricultural Faculty*, 2(1): 79-88.

Grasser, E. K., Miles-Chan, J. L., Charrière, N., Loonam, C. R., Dulloo, A. G. and Montani, J. P. (2014). Energy drink consumption in Europe: A review of the risks, adverse health effects, and policy options to respond. *Frontiers in Public Health*, 2: 134.

Karaoğlu, M. M., Kotancılar, H. G. and Aktaş, N. (2006). Effect of different modification techniques on the physicochemical and thermoanalytical properties of wheat and corn starch. *Journal of Tekirdag Agricultural Faculty,* 3(2): 83-91.

Köse, D. A. and Peker, Z. (2023). Content analysis of locally marketed energy drinks: Turkish market. *Hacettepe Journal of Biology and Chemistry*, 51(4): 341-349.

Malik, V. S. and Hu, F. B. (2010). Sugar-sweetened beverages and health: Where does the evidence stand? *American Journal of Clinical Nutrition*, 94(5): 1161–1162.

Seifert, S. M., Schaechter, J. L., Hershorin, E. R. and Lipshultz, S. E. (2011). Health effects of energy drinks on children, adolescents, and young adults. *Pediatrics*, 127(3): 511-528.

Silva, A. C., Monteiro, M. R. and Barbosa, L. F. (2021). Quantitative assessment of sugars in soft drinks using HPLC: Implications for labeling accuracy and consumer awareness. *Food Chemistry,* 342: 128321. https://doi.org/10.1016/j.foodchem.2020.128321

T. C. Resmî Gazete (2017). Turkish Food Codex Energy Drinks Communiqué No: 2017/4, June 30. *Resmî Gazete* (Issue 30110).

Zhang, Y., Müller, T. and Richter, M. (2023). Undeclared sugar content in energy drinks marketed in the European Union: A regulatory gap analysis. *Journal of Food Composition and Analysis,* 117: 105153. https://doi.org/10.1016/j.jfca.2023.105153

Zuo, J., Geng, S., Kong, Y., Ma, P., Fan, Z., Zhang, Y. and Dong, A. (2023). Current progress in natural deep eutectic solvents for the extraction of active components from plants. *Critical Reviews in Analytical Chemistry*, 53(1): 177-198.