



## Research article

# Physicochemical properties of and volatile compounds in riboflavin fortified cloudy apple juice; study of its effect on job fatigue among Egyptian construction workers



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## ABSTRACT

Fatigue and rapid exhaustion are common complaints among construction workers, as a result of high-effort levels, physical overexertion, weather and long physically demanding work hours. This study aimed to fortify cloudy apple juice with riboflavin (vitamin B2) to evaluate changes in chemical composition, antioxidant activity and volatile compounds in the fortified juice and to study its effect on the volunteer construction workers complaining of fatigue resulting from demanding physical duties. Analysis of volatile compounds in the fortified cloudy apple juice using Gas Chromatography and Gas Chromatography-Mass Spectrometry identified thirty-four volatile compounds including esters, alcohols, aldehydes and acids. The most predominant volatile compounds were alcohols followed by esters in both the control and fortified samples. We studied the effect of the supplementation of riboflavin-fortified cloudy apple juice versus conventional cloudy apple juice on the anthropometric parameters, the scores of two fatigue questionnaires (Checklist Individual Strength and Fatigue Severity Scale) and antioxidants biomarkers among young Egyptian male construction workers. This study revealed that consumption of 1.3 mg of riboflavin-fortified cloudy apple juice per day for twenty-eight days significantly improved their metabolism, with a decrease in mean body fat percentage and an increase in body muscle mass without statistically significant differences, the fortified juice significantly improved the fatigue questionnaires' scores. Moreover, the fortified supplement had a substantial change in antioxidant activity; there was significant increase in the plasma total antioxidant capacity (+74.19 %change) and catalase enzyme (+54.65 %change) with a significant decrease in the serum malondialdehyde level (-53.78 %change). When compared to the administration of conventional cloudy apple juice, although there was a significant decrease in serum malondialdehyde level (-4.63 %change) at the end of the study, only the subjective fatigue subscale of the CIS fatigue score significantly decreased among the construction workers (-24.61 %change). It could be concluded that vitamin B2 fortified-cloudy apple juice was effective in the reduction of fatigue and exhaustion in the study's subjects.

## 1. Introduction

Fatigue, a subjective term characterized by physical exhaustion with mental tiredness, and rapid exhaustion are common complaints after a long hard day at work (Wan et al., 2017). Physical fatigue refers to the decrease in muscle performance and is defined as a decrease in power or force needed in response to contractile

movement and action. Mental fatigue represents a decline in cognitive functions such as concentration, thinking, learning, and rapid response (Abd El-Fattah et al., 2015).

Fatigue affects construction workers' performance and quality of work. It manifests primarily as fast unexplained generalized muscle pain, bony pain, difficulty concentrating, cognitive dysfunctions, disturbed sleep patterns and quality resulting in daytime sleepiness, headaches or

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migraines of a new pattern or severity, feeling a sense of sadness, and a decreased productivity rate. Furthermore, a prolonged state of fatigue and/or exhaustion may result in sick leave and work disability (Aryal et al., 2017). Rapid tiredness and exhaustion are important issues in the field of applied occupational medicine that need to be considered in order to understand the link between fatigue and employee health, performance, and safety (Bazazan et al., 2014).

Construction workers or any workers with physically demanding jobs require more energy and oxygen consumption than office workers (Sabitoni, 2019). Chronic physical exertion is accompanied by an increased production of oxygen free radicals and the generation of oxidative stress with changes in serum antioxidant levels (Radak et al., 2013). Recently, there has been an increase in demand for functional foods and manufactured vitamins and minerals supplementations to decrease oxidative stress, according to people's health culture (Hadi et al., 2017). Non-steroidal anti-inflammatory drugs are frequently used to relieve pain and fatigue, despite their undesirable serious side effects such as renal damage and gastric ulcerations (Fokunang et al., 2018).

Nowadays, the demand for functional drinks and food has increased due to their benefits in reducing the risk of many diseases and conferred health benefits (Yepez et al., 2019). Apple juice can be prepared either clarified with a light brown and amber-like color, or cloudy apple juice with significant particles responsible for cloud stability, which are acceptable due to nutritional properties mainly due to the fact that they are a fresh product (Huang et al., 2012; Housseiny et al., 2013).

Apple juices are rich in polyphenol oxidase and pectinases enzymes, even after thermal interference at high temperatures to inactivate microbial contamination (Murtaza et al., 2020). During juice preparation with thermal treatments, various reactions like the Maillard reaction and oxidation cause a significant loss or degradation of aroma compounds and/or off-flavor formation during storage (Kebede et al., 2020). When apple juice was made from concentrate a significant reduction in esters occurred if re-aroma is not effective as mentioned by Chitarrini et al. (2020). Losses of eight odor-active volatile compounds occur after the processing of apple juice using a vacuum drum or ultrafiltration (Wibowo et al., 2019). In addition, there is a loss of about 33.3–59.9% in ethyl 2-methyl butyrate and 55.3–75.4% in hexanal as reported by Komthong et al. (2007) during the processing of apple juice concentrate. On the other hand, an increase in ketones and alcohols was observed in processed apple juice according to multiple studies (Schmutzer et al., 2014; Biasoto et al., 2015). Su and Wiley (2006). The change in six-active volatile compounds during processing fresh apple juice were observed and the change in these compounds were found to depend on the condition and stage of processing as well as the temperature used in pasteurization to inactivate the enzymes.

Balanced diets usually supply adequate riboflavin levels (1.3 mg/day) for an adult as recommended by the European Food Information Council (2006). Daily intake of a B2-fortified diet or beverage is recommended for specific groups. Its deficiency results from either consumption of inadequate food vitamin B2 sources or poor absorption. B2 deficiency can manifest as anemia, feet burning sensation, and loss of hair (Byrd-Bredbenner et al., 2014; Csapó et al., 2017). The previous studies showed that most apple varieties with or without apple peel contain about 0.01 mg B2/100 g fresh fruits and ranged from 0.33–2.6 µg B2/100 mL juice, which is significantly lower than other B2 sources such as chicken liver, beef kidney, milk, and dairy products (Zandomenighi et al., 2007; Loncari et al., 2020).

The study aimed to evaluate the effect of the fortification of cloudy apple juice with B2 on physicochemical properties and volatile compounds, and to study its impact on the volunteer construction workers complaining of fatigue and exhaustion due to their physically demanding work which was relieved by rest or sleep.

## 2. Materials and subjects

### 2.1. Materials

#### 2.1.1. Fruit collection and preparation of cloudy apple juice

*Malus domestica* Boekapples (Var Anna) at commercial maturity was purchased from the local market during autumn (October–November) of 2020. Fruits were carefully selected for uniformity in maturity, colour and absence of physical damage. After cleaning the apple with tap water, they were cut into halves and put into juice extractors to press the apple using (Multipress automatic Braun MP80, Kronberg, Germany). 1 g L<sup>-1</sup> ascorbic acid was added to the pressed juice to avoid disagreeable enzymatic browning. Then, the obtained samples were filtered through a 4-layer cheese cloth and poured into beakers containing vitamin B2 at a level of 1.3 mg/240 mL juice. Vitamin B2 (riboflavin) yellow powder raw material API CAS 83-88-5 was obtained from Fengchen Group Co. Ltd. (Qingdao Fengchen Technology and Trade Co., Ltd.) in Qingdao, China. The sample without adding B2 was considered the control. The fortified samples and the control were stored for 3 days in a refrigerator.

#### 2.1.2. Headspace Gas Chromatography-Mass Spectrometry (GC-MS) analysis

Headspace-solid phase micro-extraction (HS-SPME) procedure was conducted in accordance with Kaprasob et al. (2017). In a 20 mL vial sealed with a PTFE/silicone-coated septum, 10 mL aliquot of juice was added to 3 g NaCl. HS-SPME was used to extract the volatile compounds. Before fiber exposure, the vial was submerged in a water bath at 40 °C for 15 min. The fiber was then exposed to the sample headspace for 60 min under constant stirring at the same temperature, after which it was submitted for GC analysis. The fiber was desorbed at 250 °C for 10 min at the injector port, set in split less mode for 2 min. The compounds were separated on a ZB-WAX Plus polar phase capillary column (Zebron, Phenomenex, USA; 60 m × 0.25 mm × 0.25 µm). Helium was used as carrier gas, with an initial flow of 1 mL min<sup>-1</sup>. The initial column temperature was adjusted to 35 °C for 3 min, followed by an increase of 2 °C per minute to 80 °C and finally an increase of 5 °C per minute to 230 °C while maintaining isothermal conditions for 5 min. The temperature in the detector was maintained at 230 °C. A series of homologous n-alkanes were analyzed under the same conditions to calculate the linear retention index (LRI) for analyte identification. Electron ionization at 70 eV was used to obtain the mass spectra with a scanning range of 35–400 m/z. The mass spectrometry ion source and MS quadrupole temperature was set at 230 °C and 150 °C, respectively. The volatile compounds identification was carried out by comparing experimental mass spectra with those from National Institute of Standards and Technology library (NIST 05) and the experimental linear retention index (LRI), which was calculated as follows:

$$I_x = 100 \left[ \frac{(t_x - t_n)}{(t_{n+1} - t_n)} + n \right]$$

where  $I_x$  is the temperature-programmed retention index,  $t_n$ ,  $t_{n+1}$  and  $t_x$  are the retention times (in minutes) of the two n-alkanes containing n and n + 1 carbons and of the compound of interest, respectively. The obtained values were compared with those in the literature (Adams, 2007).

#### 2.1.3. Sensory evaluation

The conventional cloudy apple juice and the one fortified with riboflavin were provided based on the duo-trio method to the volunteers in clean, transparent 240 mL plastic cups to evaluate the following attributes before their consumption: appearance, aroma, taste, texture, color, and overall acceptability. Appearance, aroma, taste, texture, colour, and overall acceptability were evaluated on a 9-Point Hedonic scale according to Aguiar et al. (2012). Mineral water was used by the volunteers to rinse their mouths between samples.

#### 2.1.4. Total soluble solids, titratable acidity, and pH measurements

The total soluble solids (TSS) and the titratable acidity (TA) were determined according to AOAC (2016) and results were expressed as g

maleic acid 100 per milliliter sample. The pH was measured in each sample using a pH meter (Hanna pH-meter HI 9021 m Germany) according to AOAC (2016) methods.

### 2.1.5. Colour measurements

Objective evaluation of juice sample colours were measured by Hunter L\*, a\*, and b\* parameters using a spectro-colorimeter (Tristimulus Colour Machine) in accordance with Sapers and Douglas (1987).

### 2.1.6. Determination of total phenolic contents, phenolic compounds, total flavonoid content, and antioxidant activity

The total phenolic content was assessed in accordance with Zilic et al. (2012); the result was presented as [ $\mu\text{g}$  of gallic acid equivalent (GAE) per g of sample]. The phenolic compounds extracted in both the control cloudy apple as well as the fortified juices were detected by HPLC as described by Alberti et al. (2014). Peaks of the compounds were identified and quantified by comparing the retention times and spectra of the samples with external standards that had been previously prepared. The total flavonoid ABTS radical scavenging content was evaluated in accordance with Chandra et al. (2014) and the result was expressed as mg ceftiofur equivalent (CE)/mL. The antioxidant activity of the two prepared juice samples was evaluated by assay in accordance with Hwang and Thi (2014). The result was presented as mg Trolox equivalent (TE)/mL sample.

## 2.2. Subjects

This study was conducted at the Nutrition Department of NRC - Egypt, in October and November to avoid the hot weather of the summer which may have had a negative effect on the physical performance of the construction workers. Eighty-four young Egyptian male construction worker volunteers reporting fatigue that was relieved by rest and sleep were included in the twenty-eight day study. The protocol of the study was approved by the NRC Ethics committee (registration number: 19/178) and signed written informed consent forms from each of the male volunteers were required for participation in the research project following a full explanation of the study.

Subjects were young Egyptian male construction workers who had normal body mass index numbers, were of similar socioeconomic status, complained of fatigue (diagnosed by the calculated Fatigue Severity Scale total score  $\geq 36$ ) due to physically demanding job (8–10 working hours/day for 6 days/week), experienced fatigue relieved by rest or sleep, and were within the age range of 21–30 years. Before starting the study, the subjects were clinically examined to check their medical status. The selection of participants was based on the absence of any acute or chronic illness. They did not suffer from any medical diseases (hypertension, diabetes mellitus, anemia, thyroid dysfunctions, dyslipidemia, bone or joints diseases and no renal or liver diseases), they had normal 25 Hydroxy Vitamin D level, and no history of antidepressants or antipsychotic drug therapy. Any subject who had medical diseases, obesity, or was receiving vitamin supplements was excluded from the study. We advised the subjects not to consume analgesics, non-steroidal anti-inflammatory drugs, vitamins, minerals, or any pharmacological supplements during the study period, which lasted for 28 days. Moreover, no changes in their nutrition habits were implemented.

The participants were divided into two groups; group (A) who had a daily consumption of 240 mL of riboflavin-fortified cloudy apple juice and group (B) who had a daily consumption of 240 mL of conventional cloudy apple juice as a control group. All the previously mentioned parameters were performed for each subject twice; at the start of the study (1<sup>st</sup> day) and at the end of the study (28 days later).

### 2.2.1. Assessment of anthropometric parameters

Relevant anthropometric measurements were assessed. Height, weight, and body mass index (BMI) were calculated (weight kg/height<sup>2</sup> meter), and percent body fats (%BF), muscle mass and BMR were

reported using Geratherm Body Fitness (B-5010), Germany. Blood pressure and heart rate were recorded.

### 2.2.2. Assessment of fatigue by questionnaires

**2.2.2.1. Fatigue Severity Scale (FSS).** The FSS was evaluated on the first visit for all subjects, to evaluate the subjective condition of “rapid exhaustion and fatigue during work which was relieved by rest” to select the candidates for the study. The FSS consisted of 9 short questionnaires that were used to assess the severity of the fatigue and how much it affected the subject's activities and daily life e.g. (motivation decreases when he is exhausted; he is more easily fatigued than before; fatigue interferes with his physical performance; fatigue interferes with his work, etc.). Total score was calculated and a calculated score  $\geq 36$  suggested that subject suffers from fatigue (Valko et al., 2008).

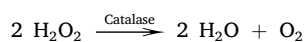
**2.2.2.2. Checklist Individual Strength (CIS).** The CIS consists of 20 simply worded questionnaires, divided into four components: subjective practice of fatigue subscale, decreased concentration subscale, decreased motivation subscale, and decreased physical activity level subscale. Each questionnaire has a seven-point rating scale [ranging from 1 (i.e. yes it is true) to 7 (i.e. no it is not true)]. The CIS proved to be highly reliable with a cutoff score  $\geq 35$  being considered high. The questionnaire is considered appropriate for working persons (Vercoulen et al., 1999; Aratake et al., 2007).

### 2.2.3. Biochemical analysis

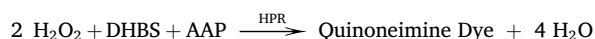
Blood samples (5 mL) were drawn and were allowed to clot, then centrifuged to separate the sera which were divided into aliquots and stored in Eppendorf tubes at ( $-70\text{ }^{\circ}\text{C}$ ) until they were used for further analysis.

**2.2.3.1. Determination of Malondialdehyde level.** Malondialdehyde (MDA) level was determined by the method of Satoh (1978). MDA can react with thiobarbituric acid (TBA) in an acidic medium at a temperature of  $95\text{ }^{\circ}\text{C}$  for 30 min to form a coloured complex, which can be measured colourimetrically. The concentration of the thiobarbituric acid reactive substances was calculated by the absorbance coefficient of malondialdehyde thiobarbituric acid complex at 534 nm (Satoh, 1978).

**2.2.3.2. Determination of catalase enzyme.** Catalase is an antioxidant enzyme that presents in most aerobic cells. It serves as one of the body's defense systems against a strong oxidant ( $\text{H}_2\text{O}_2$ ) that can cause intracellular injury and/or damage. Catalase assay adds to the family of antioxidant biomarkers and provides another useful tool for oxidative stress investigations (Aebi, 1984).



In the presence of peroxidase (HPR), remaining  $\text{H}_2\text{O}_2$  reacts with 3,5-Dichloro-2-hydroxybenzene sulfonic acid (DHBS) and 4-aminophenazone (AAP) to form a chromophore with a color intensity inversely proportional to the amount of catalase in the original sample (Aebi, 1984).



**2.2.3.3. Determination of total antioxidants capacity.** The serum total antioxidant capacity (TAC) was determined according to the colorimetric method of Koracevic et al. (2001). The assay measured the capacity of the biological fluids to inhibit the production of thiobarbituric acid reactive substances (TBARS) from sodium benzoate under the influence of the free oxygen radicals derived from Fenton's reaction. A standardized solution of Fe-EDTA complex reacts with hydrogen peroxide through a Fentontype reaction, leading to the formation of hydroxyl radicals (OH).

These reactive oxygen species degrade benzoate, resulting in the release of TBARS. Antioxidants from the added sample of human fluid cause suppression of the production of TBARS. This reaction can be measured spectrophotometrically at 532 nm.

### 2.3. Statistical analysis

Data were presented as the mean  $\pm$  Standard Deviation (SD) values. One way analysis of variance (ANOVA) was carried out, and the statistical comparisons among the groups were performed with Post Hoc and the least significant difference (LSD) tests using a statistical package for social science. Pearson's correlation was also used for statistical analyses by SPSS Statistics ver. 16 software (SPSS Inc. Chicago, IL, USA).  $P < 0.05$  was considered as statistically significant.

## 3. Results and discussion

### 3.1. Effect of B2 on volatile constituents in cloudy apple juice

The perception of apple fruits and apple products' flavor results from a mixture of various volatile components including alcohols and esters (Espino-Díaz et al., 2016). The analysis of volatile compounds in cloudy apple juice, odour threshold values, as well as aroma description are

shown in Table 1; thirty-one volatile compounds have been identified and were classified into four groups including: esters, alcohols, aldehydes, and acids. The esters (19) were the largest group of volatile compounds followed by alcohols (9), aldehydes (3) and acids (3). The data in (Table 1) exhibited that alcohols particularly ethanol (20.16%) and 2-butanol (12.49%), followed by esters, were the most predominant volatile compounds in both the control and the B2 fortified-sample. The aroma of apple is imparted from even a small concentration of alcohols, which may be used as a suitable solvent for other aromas (Laaksonen et al., 2017). In the present study, nine alcohols were detected as the main volatile compounds in cloudy apple juice. The most common alcohol in the investigated sample was ethanol with concentrations of 20.16% and 20.28% in the control and the fortified juice, respectively. The concentrations of detected volatile compounds were higher in the fortified apple juice compared to the control. Nineteen kinds of esters were detected and the main type was ethyl acetate, which represented 11.34% and 12.45% in the control and the fortified sample at zero time, respectively. Hexyl acetate (sweet, fruity) was the second highest concentration in both the control and the fortified juice, representing 8.65% and 8.79%, respectively. These data are in agreement with Ferreira et al. (2009) who found that the most common volatile compounds in Ponta do Pargo apple were  $\alpha$ -farnesene (30.49%), and hexyl acetate (6.57%). Also, Kebede et al. (2018) identified ten esters using headspace in apple juice

**Table 1.** Effect of riboflavin and storage on aroma volatile compounds of cloudy apple juice.

Volatile compounds	KI <sup>a</sup>	Control		B2 fortified		OTH (ug/L) <sup>c</sup>	Aroma description <sup>d</sup>
		Fresh	Stored	Fresh	Stored		
Methyl acetate	895	0.21 <sup>b</sup>	0.91	0.26	0.24	8300	
Ethyl acetate	926	11.43	16.28	12.45	12.39	5000	Paint, fruity
Ethyl propanoate	971	2.08	0.80	2.09	1.95		fruity
Methyl butanoate	986	0.08	0.17	0.20	0.21	5	Fruity, sweet
Methyl 2-methylbutanoate	991	0.64	0.46	0.76	0.63		Sweet caramel, grape
Methyl 3-methylbutanoate	1003	1.07	n.d	1.20	1.16		
Ethyl butanoate	1027	n.d	n.d	0.12	0.87	1	Fruity, apple
Ethyl 2-methylbutanoate	1042	1.05	0.21	1.17	1.02	0.00006	
Ethyl 3-methylbutanoate	1059	2.46	0.24	2.58	2.34		
Butyl acetate	1067	6.74	19.82	6.86	6.62	10	Apple-like, fresh, fruity
Ethyl pentanoate	1129	0.63	1.39	0.75	0.51	0.005	
Propyl propanoate	1158	3.47	5.42	3.60	3.36		
Methyl hexanoate	1183	1.38	3.15	1.50	1.26		
Ethyl hexanoate	1235	3.55	0.27	3.67	3.43	1	Apple peel, fruity
Hexyl acetate	1273	8.65	12.04	8.79	18.72	2	Sweet, fruity
Ethyl 3-hexenoate	1297	0.11	n.d	0.23	0.64		
Hexyl propanoate	1335	0.46	0.32	0.58	0.34		
Ethyl 2-hexenoate	1339	0.72	0.50	0.84	0.92		
Hexyl butanoate	1534	n.d	n.d	0.12	0.34	250	
Ethanol	953	20.16	15.02	20.28	8.45	100000	Sweet
1-Propanol	1025	1.23	2.74	1.35	1.12	9000	
2-Butanol	1143	12.49	0.20	12.61	12.37	5000	Green, wine
3-Methyl-1-butanol	1205	8.25	10.88	8.37	11.24		
1-Hexanol	1356	2.62	1.69	2.74	2.50	150	
1-Octen-3-ol	1442	0.12	0.58	0.25	0.53		
Butanal	923	0.18	1.47	0.07	0.15		Apple
2-Methylbutanal	942	0.23	0.26	0.15	0.06		
Hexanal	1074	1.34	1.37	0.46	1.02	5	Grass, tallow, fat
Acetic acid	1446	0.85	0.11	0.97	0.97		Acid
Hexanoic acid	1817	1.22	0.59	0.56	0.76	3000	
Octanoic acid	2125	0.15	0.08	0.27	0.56	3000	

<sup>a</sup> Kovats index.

<sup>b</sup> Values are expressed as area percentage.

<sup>c</sup> OTH: Odor threshold values cited from Anesea et al. (2020); Pino and Quijano (2012).

<sup>d</sup> Odour description cited from (Kebede et al., 2020; Komthong et al., 2007).



that were stable after pasteurization and pulsed electric field processing and mentioned that ethyl acetate plays a role in apple juice aroma.

Generally, esters were the most common volatile compounds in apple juice responsible for the fruity and sweet odour derived from fatty acids (Brackmann et al., 1993; Both et al., 2014). Esters, especially hexyl acetate, butyl acetate and 2-methyl butyl acetate, had a substantial impact on apple flavor, as mentioned by (Raffo et al., 2009; Iglesias et al., 2012).

The obtained results showed that fortification of cloudy apple juice with riboflavin resulted in the retention of the main volatile compounds, especially esters and alcohols, during storage. Our data is in accordance with Wu et al. (2020) who found that lactic acid bacteria (six strains) maintain the volatility of apple juice during fermentation. The low concentrations of aldehydes in the current study may be due to the full ripening and maturation of the fruits used in this investigation. Several studies have stated that aldehydes could cause an undesirable taste in apple juice (Cousin et al., 2017). C6 alcohols and aldehydes in the juice fortified with B2 were higher than in the control, including 1-Hexanol and hexanal. The aroma of volatile compounds depends on their concentration as well as the odour threshold value. Table 1 showed the threshold values of some of the identified volatile compounds. Alcohols were present in much higher concentrations than aldehydes and esters.

### 3.2. Sensory evaluation

The sensory description of riboflavin-fortified apple juice are shown in Table 2, the fortified juice did not differ significantly from conventional cloudy apple juice and was found highly acceptable by the subjects.

### 3.3. Physicochemical properties

The physicochemical properties of the fresh cloudy apple juice as well as the sample of the one fortified with riboflavin were determined and the data is provided in Table 3. During fortification, there was a slight increase in total soluble solids and an insignificant decrease in pH and acidity. Our results agree with Kelebek et al. (2009). The acidity in apple juice expresses the content of various organic acids such as citric, shikimic, quinic, etc. and the most predominant is malic acid (Wu et al., 2007). Acidity and pH play an important role in the acceptability of apple and apple products by consumers. The color quality of riboflavin-fortified apple juice exhibited significant changes in parameters determined as a and b-values, particularly impacting the a-value which indicated a yellow color was found in the fortified sample.

### 3.4. Total phenolic contents, total flavonoid contents, and antioxidant activity of the supplemented juices

Table 4 demonstrated that B2-fortified apple juice fortified had potent antioxidant activity as it had higher ABTS radical scavenging activity and higher total phenolics and flavonoids content than the conventional cloudy apple juice. The results given in Tables 4 and 5 indicated that fortified apple juice was five times richer than the control juice in total phenolic contents, 2.14 versus 0.37 (mg GAE/mL) respectively, with an average of phenolic compounds ranging from 3.03 to 17.35 (ug/mL). Phenolic compounds were mainly Caffeic, Syringic, Catechin and

Table 2. Sensory evaluation of the supplemented juices.

Samples	Appearance	Taste	Aroma	Texture	Colour	OAA
Control	9	9	9	9	9	9
Apple + B2	8.7 ± 0.72	8.4 ± 0.67	8.6 ± 0.32	8.2 ± 0.57	8.9 ± 0.42	8.9 ± 0.38

OAA: overall acceptability.

Table 3. Physicochemical properties of the supplemented juices.

Samples	TSS	pH	Acidity	L	a	b
Control	14.05 ± 0.35 <sup>a</sup>	3.57 ± 0.08 <sup>a</sup>	3.42 ± 0.31	22.08 ± 0.11	10.96 ± 0.45	23.18 ± 0.15
Fortified	14.31 ± 0.12 <sup>a</sup>	3.61 ± 0.18 <sup>a</sup>	2.97 ± 0.29	19.56 ± 0.16	8.43 ± 0.29	24.19 ± 0.27

TSS: Total soluble solids. Values are expressed as (Mean ± SD). Different letters in the same column are significantly different.

Table 4. Total phenolic contents, total flavonoid contents, and antioxidants activity of the supplemented juices.

Sample	Total phenolics (mg GAE/mL)	Total flavonoids (mg CE/mL)	ABTS (mg TE/mL)
Control	0.36 ± 0.001	0.11 ± 0.01	0.38 ± 0.003
fortified cloudy apple juice	2.14 ± 0.002	0.84 ± 0.02	1.10 ± 0.001

ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) an antioxidant activity assay.

Table 5. Effect of riboflavin fortification on phenolic compounds (ug/ml) of the cloudy apple juice.

Compound (ug/ml)	Conventional cloudy apple juice	B2 fortified cloudy apple juice
Protocatechuic	ND	ND
p-hydroxybenzoic	0.47	ND
Catechin	ND	6.28
Chlorogenic	0.56	ND
Caffeic	ND	15.37
Syringic	1.32	9.60
Vanillic	ND	ND
p-coumaric	5.47	ND
Rosmarinic	0.79	ND
Apigenin-7-glucoside	0.53	3.03

Apigenin-7-glucoside, in descending order. Caffeic acid is used in supplements to boost athletic performance, exercise-related fatigue, weight loss, and in treatment of cancer, HIV/AIDS, herpes, and other conditions (Yilmaz, 2019). Syringic acid (SA) is a phenolic compound of natural origin. SA is an excellent compound to be used as a therapeutic agent for various diseases (diabetes, cardiovascular diseases, cancer, cerebral ischemia, and liver damage) and it possesses anti-oxidant, antimicrobial, anti-inflammatory, and antiendotoxic activities (Cheemanapalli et al., 2018).

Apple, when juiced, is a super food as it supports hydration and human health (Hyson, 2011). Apple, like most fruits and vegetables, contains phenolic compounds, bioactive phytochemicals which play an important role in health. These phenolic compounds slow down free radical production and the oxidative sequence reaction (Gorinstein, 2009). The phytochemical composition of apples varies significantly depending on the varieties of apples, their maturation, ripening, and processing. Apples contain quercetin, a flavonoid which has anti-inflammatory and antioxidant effects (Batiha et al., 2020). Storage had little effect on phytochemical activities (Boyer and Liu, 2004). Candrawinata et al. (2012) concluded that the clarification process of apple juice minimized its phenolic content and its antioxidant activity and that the cloudy variety is richer in antioxidant activity and may be used in the food industry. Rydzak et al. (2020) demonstrated that commercial cloudy apple juice had a total phenolic content three times higher than clear apple juices.

### 3.5. Results of the effect of juice supplementation on fatigue scores, anthropometrics parameters, and biochemical data among the studied groups

Fifty-seven volunteer construction workers started the study, fifty-four of them completed the study. They were smokers with a primary-level education and belonged to the same low socioeconomic level. Thirty-one participated in group (A) with a mean age of  $24.42 \pm 2.32$  years while twenty-three subjects participated in control group (B) with a mean age of  $24.43 \pm 1.95$  years. All subjects worked daily (except on Friday) from 7 AM to 5 PM, with a 1-h break per day. The subjects sometimes needed to also work on Fridays to compensate for previous work delays. Data presented as mean and SD for relevant anthropometric measurements, calculated fatigue scores and biochemical parameters before and after juice interventions in both groups are shown in Table 6. All parameters and scores for both groups were comparable at baseline. After twenty-eight days of intervention, the participants in group (A) consuming the riboflavin-fortified cloudy apple juice supplement showed a statistically significant increase in their basal metabolic rates (increased basal metabolic rate means an increase of the number of calories burned at rest), improvement in total CIS score, subjective fatigue subscale and physical activity subscale, FSS score, MDA, catalase enzyme, and total antioxidant capacity serum levels. Their mean percentage of body fat decreased numerical and body muscle mass increased but without statistical significance. While subjects in group (B) consuming cloudy apple juice showed a significant decrease in the subjective fatigue subscale of the CIS score and MDA serum level. In comparing the results from both groups, there appeared to be a significant difference between the two juice supplements in their effect on BF, BMR, body muscle mass, the two evaluated fatigue scales, and antioxidants parameters. More improvement occurred in the fortified cloudy apple juice supplement group. There was no effect on heart rate or blood pressure in either group. Vitamin B2 has an antioxidant activity that helps the body deal with oxidative stress. The correlation between the total CIS score and FSS score with different parameters at baseline was tested using the Pearson test and is demonstrated in Table 7. Fatigue scores had significant direct

**Table 7.** Pearson Correlation between CIS and FSS scores and different variables in the two groups at the start of the study.

	Total CIS	FSS
Body mass index	.067	.226
Percentage body fat	.520**	.954**
Muscle mass	-.540**	-.962**
Basal metabolic rate	-.533**	-.976**
Systolic blood pressure	.030	.029
Diastolic blood pressure	.064	.102
Heart rate	.333**	.629**
Total antioxidant capacity	-.476**	-.888**
Catalase enzyme	-.485**	-.956**
Malondialdehyde	.502**	.965**

CIS: Checklist Individual Strength; FSS: Fatigue Severity Scale; numbers presented in this table are the value of  $r$  = correlation coefficient. \*\*Correlation is significant at the 0.01 level (2-tailed).

correlations to body fat percentages, and HR and MDA levels, and there was a negative correlation to body muscle mass, BMR, serum catalase and TAC levels. Otherwise, fatigue scores were not significantly related to BMI and blood pressure.

Heavy hard work induced muscle fatigue which differs from chronic fatigue syndrome CFS in the resting stage. Fatigue of CFS occurs prior to any activity or exercise, while fatigue due to hard work is caused by prolonged physical activity inducing muscle fatigue and is defined as a reversible loss of muscle contractility during work which is relieved by rest and/or sleep (Taghizadeh et al., 2016; Wan et al., 2017). Finsterer (2012) concluded that biomarker assessment to quantify the severity of muscle fatigue during physical activity is mandatory, as oxidative stress increases during physical activity and results in fatigue and decreased performance. No laboratory biomarkers or biochemical parameters are available for the assessment of fatigue, however Zhang et al. (2015) declared that fatigue could be estimated through questions to which the workers give their opinion about their level of fatigue (Zhang et al.,

**Table 6.** Mean  $\pm$  SD of the studied clinical and biochemical parameters among the participants at the basal and last visit of the study.

Parameters	Group A (no. = 31) Riboflavin fortified cloudy apple juice			Group B (no. = 23) Cloudy apple juice		
	Day 1	Day 28	% change	Day 1	Day 28	% change
	Mean $\pm$ SD			Mean $\pm$ SD		
Age (years)	24.42 $\pm$ 2.32			24.43 $\pm$ 1.95		
BMI (kg/m <sup>2</sup> )	26.11 $\pm$ 1.02	26.09 $\pm$ 1.00	-0.08	25.59 $\pm$ 0.85	25.59 $\pm$ 0.98	0.0
SBP (mm Hg)	114.35 $\pm$ 6.92	114.03 $\pm$ 7.24	-0.28	114.57 $\pm$ 6.73	113.91 $\pm$ 6.73	-0.58
DBP (mm Hg)	61.77 $\pm$ 3.04	61.61 $\pm$ 3.26	-0.26	61.52 $\pm$ 3.51	61.52 $\pm$ 3.51	0.0
HR (beats/min)	70.13 $\pm$ 4.30	70.26 $\pm$ 4.31	0.19	69.57 $\pm$ 4.34	69.48 $\pm$ 4.06	-0.13
BF (%)	15.99 $\pm$ 1.96	15.87 $\pm$ 1.92	-0.75	15.09 $\pm$ 1.8	15.09 $\pm$ 1.9	0.0 <sup>a,c</sup>
Muscle mass (Kg)	50.26 $\pm$ 4.75	50.28 $\pm$ 4.63	0.04	49.85 $\pm$ 4.8	49.82 $\pm$ 4.7	-0.06 <sup>a,c</sup>
BMR (kcal/day)	2824.74 $\pm$ 143.99	2956.71 $\pm$ 139.01 <sup>a</sup>	4.67	2899 $\pm$ 149.87	2907.2 $\pm$ 150.6	0.28 <sup>a,c</sup>
Total CIS	91.81 $\pm$ 5.29	31.84 $\pm$ 3.01 <sup>a</sup>	-65.32	76.74 $\pm$ 6.27	63.57 $\pm$ 5.67	-17.16 <sup>a,c</sup>
Subjective fatigue subscale	52.84 $\pm$ 3.40	14.16 $\pm$ 2.72 <sup>a</sup>	-73.20	40.83 $\pm$ 5.98	30.78 $\pm$ 5.61 <sup>a,b</sup>	-24.61 <sup>a,c</sup>
Decreased Physical activity subscale	20.19 $\pm$ 1.05	3.58 $\pm$ 0.67 <sup>a</sup>	-82.27	20.04 $\pm$ 1.10	18.74 $\pm$ 1.09	-6.49 <sup>a,c</sup>
Decreased motivation subscale	8.84 $\pm$ 3.21	5.58 $\pm$ 1.11	-36.88	5.91 $\pm$ 1.31	5.52 $\pm$ 1.08	-6.60 <sup>a,c</sup>
Decreased concentration subscale	9.94 $\pm$ 1.73	8.52 $\pm$ 1.46	-14.29	9.96 $\pm$ 1.87	8.52 $\pm$ 1.50	-14.46
FSS	42.71 $\pm$ 4.86	13.61 $\pm$ 3.91 <sup>a</sup>	-68.13	41.83 $\pm$ 3.93	38.61 $\pm$ 4.19	-7.70 <sup>a,c</sup>
Catalase (U/L)	52.34 $\pm$ 8.69	80.94 $\pm$ 7.60 <sup>a</sup>	54.65	53.32 $\pm$ 9.35	54.04 $\pm$ 7.90	1.35 <sup>a,c</sup>
TAC (mM/L)	0.62 $\pm$ 0.11	1.08 $\pm$ 0.60 <sup>a</sup>	74.19	0.59 $\pm$ 0.10	0.60 $\pm$ 0.09	1.69 <sup>a,c</sup>
MDA (nmol/ml)	5.82 $\pm$ 1.10	2.69 $\pm$ 0.63 <sup>a</sup>	-53.78	6.05 $\pm$ 1.14	5.77 $\pm$ 1.12 <sup>a,b</sup>	-4.63 <sup>a,c</sup>

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; BF: body fat; BMR: basal metabolic rate; CIS: Checklist Individual Strength; FSS: Fatigue Severity Scale; TAC: total antioxidant capacity; MDA: malondialdehyde; a & b: Before vs. After of group A and group B respectively. c: % changes in group A vs. group B. Significant at \* $p < 0.05$ .

2015). Checklist Individual Strength and Fatigue Severity Scale questionnaires are suitable tools for measuring fatigue in the working population (Bültmann et al., 2000). CIS is capable of distinguishing between fatigued and non-fatigued employees in occupational groups (Beurskens et al., 2000). Furthermore, Techera et al. (2016) stated that there are several potential tools to assess the feeling of fatigue, including the Swedish Occupational Fatigue Inventory and Checklist Individual Strength questionnaires.

Construction workers operate heavy equipment such as concrete mixers to prepare construction sites. Awareness of the complications of long-term hard physical work is a great step towards avoiding potentially catastrophic consequences and knowing the importance of taking care of their bodies is vital (Jones et al., 2019). The ability to carry out hard physical activity depends on several factors including vitamin B1 thiamine, vitamin B2 riboflavin, and vitamin B6 pyridoxine (Eaves et al., 2016).

Riboflavin B2 is a water soluble vitamin that is needed for overall good health. It must be restored daily using dietary sources. B2 has a role in energy production, and it allows oxygen to be used by the body. It helps the body to break down carbohydrates, proteins, and fats to produce energy and plays a vital role in maintaining the body's energy supply. B2 helps convert carbohydrates into adenosine triphosphate which is vital for storing energy in muscles. B2 is utilized in the functioning of the digestive tract, blood cells, and other organs and its presence is necessary for the body to benefit from Iron, folate, homocysteine, and vitamin B6 (O'Callaghan et al., 2019; Mahabadi et al., 2020). B2 is an essential component of the coenzymes flavin mononucleotide FMN and flavin adenine dinucleotide FAD, which are crucial for energy production, cellular function, cell growth, metabolism of essential fatty acids, metabolism of medicines and steroids, the conversion of the amino acid tryptophan to niacin, the absorption of iron, the synthesis of folic acid, the synthesis of heme protein, and the regulation of thyroid hormones. They are also essential cofactors in the glutathione redox cycle (Kennedy, 2016; Said and Ross, 2013). The normal recommended daily allowance depends on age and gender (1.3 mg daily for men). A higher dose can be used to treat cataract, migraines, and headaches. Riboflavin is considered safe at high doses because excess B2 is excreted by the urinary system (Thakur et al., 2017).

#### 4. Conclusion

To date, there have been few studies addressing the health of construction workers. Nutrition is a key element to improve their strength. Fatigue can be avoided by planning programs and consuming functional foods and/or beverages that support health and wellness. Vitamin B2-fortified cloudy apple juice is a healthy beverage that contains potent antioxidants and is rich in phenol and flavonoid compounds. It is effective in the reduction of fatigue in construction workers. Vitamin B2-fortified products are recommended for those with physically demanding jobs.

#### Declarations

##### Author contribution statement

Suzanne Fouad: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Gamil E. Ibrahim: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Ahmed M. S. Hussein: Conceived and designed the experiments; Analyzed and interpreted the data.

Fatma A. Ibrahim: Performed the experiments; Analyzed and interpreted the data.

Aliaa El Gendy: Performed the experiments.

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#### Data availability statement

Data included in article/supplementary material/referenced in article.

#### Declaration of interests statement

The authors declare no conflict of interest.

#### Additional information

No additional information is available for this paper.

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