Original Article
Food Science and Technology

ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.1590/fst.105122

Evaluation of a Jordanian commercial chocolate brand fortified with micronutrients of vitamins B_{12} and D_3, iron and zinc

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Abstract

Chocolate is a preferred food for children and adults. Micronutrient deficiencies are still prevalent worldwide. This study aims to manufacture and evaluate a type of sugar-free dark chocolate fortified with vitamins and minerals. Three types of chocolate were fortified with vitamin D_3, vitamin B_{12}, and iron and zinc. The fortified chocolate was evaluated in terms of chemical composition using FTIR and DSC methods, visco-mechanical, sensory and stability. The FTIR studies indicated that the spectrum of all fortified chocolates was similar to that of the control, unfortified chocolate. The DSC studies indicated that there were no characteristics differences in crystallinity and degree of crystallization between the fortified and the control products. The addition of vitamins and minerals to chocolate strengthened the structure, increased the melting point, didn't cause significant variation in the rheological properties and had no significant effect on the overall sensation of the taste when compared with controlled chocolate. The stability results indicated that the fortified chocolate was stable for 9 months at room temperature. It was concluded that the fortified chocolate was a good vehicle for the vitamins and minerals with acceptable properties that may be used for supplementing vitamins and minerals.

Keywords: chocolate; fortified; vitamin D; vitamin B_{12}; iron; zinc.

Practical Application: The fortified chocolate was delicious, affordable and good vehicle for vitamins and minerals.

1 Introduction

Inadequate intake of the essential micronutrients; iron (Fe), zinc (Zn), and vitamins D_3 and B_{12}, is common in many countries, particularly among countries in the Middle East (Hwalla et al., 2017; World Health Organization, 2010). Micronutrients, vitamins and minerals, are substances required in minute amounts for the growth and health of the human body. Vitamins and minerals play important roles in digestion, absorption and metabolism. Their level in the blood must be kept in a certain normal range to maintain health (Rawson et al., 2020). People in developing countries usually suffer from malnutrition and micronutrient deficiencies which adversely affect the blood levels of many important vitamins and minerals (Müller & Krawinkel, 2005). The deficiency in vitamin and mineral levels is usually associated with many diseases, especially in pregnant women, infants, and young children (Gernand et al., 2016; Wagner & Greer, 2008).

A study showed that about one-third of Jordanian adults had vitamin B_{12} deficiency (< 200 µg/mL). Intake of vitamin B complex and multivitamins seems to protect from vitamin B_{12} deficiency (El-Khateeb et al., 2014). Another study found that the prevalence of vitamin B_{12} deficiency among Jordanian men and women aged 19 to 25 years was high (32.1%) (El-Qudah et al., 2013). A recent study found a correlation between geographical locations and vitamin B_{12} deficiency in Jordan. This study showed that 24.6% of the population in Aqaba city (south Jordan) had vitamin B_{12} deficiency which is lower than that of the rest of Jordan, especially in northern areas (Qar et al., 2021).

The *per capita* estimated consumption of calcium for all Jordanians was 829 mg/day. These results indicate that the intakes of calcium were moderately low (Alkurd, 2011). Takruri et al. (2011) found that Jordanians were consuming very limited servings of milk group (0.6 servings *per capita*/day) which is the main dietary source of calcium. Vitamin D potentiates the absorption of calcium and phosphate from the small intestine into the circulatory system, so, it is essential for the maintenance of bone structure and support (Rawson et al., 2020; Khuri-Bulos et al., 2014). Vitamin D deficiency has been associated with interrupted bone growth and/or ossification, growth retardation, dysfunctional calcium metabolism, and other adverse health effects (Amrein et al., 2020; Hollis & Wagner, 2005; Holick, 2008). The low levels of vitamin D and calcium intake are a double whammy to the Jordanian's bone health. Solving such a common problem requires increasing the availability of the food sources of these nutrients; vitamin D- and calcium-fortified foods are one of their direct solutions. Products to which nutrients are added are known as functional foods (Qinna et al., 2013).

Received 10 Oct., 2022
Accepted 09 Dec., 2022
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Research carried out in the years of 1990s indicated the deficiency of several vitamins in Jordanian children. This finding encouraged the Ministry of Education in Jordan to launch the pioneering School Snack Service (SSS) program for the young school children in deprived regions in Jordan (Khatib & Hijazi, 1993; Khatib et al., 1995). In 2002, the Ministry of Education sponsored a survey aimed at assessing the nutrition and growth status of young school children in underprivileged areas in Jordan. Results indicated the existence of problems regarding dietary consumption patterns. Royal Philanthropies sponsored several pioneering interventional programs such as the 'multi-vitamin tablet' for all schools in 2003, 'multi-micronutrient'- fortified biscuits for the poor school children in 2004 and the 'Wheat Flour Fortification' program in 2006 (Khatib & Hijazi, 2009).

Fortification programs usually included micronutrients of vitamins and minerals such as vitamin A, iron, folic acid, zinc, and B-vitamins (B_1, B_2, B_3, B_6, and B_{12}) (Khatib & Hijazi, 2009). Some studies showed that the problem of deficiency of some important vitamins required for infant and children's growth is still unresolved (Khatib & Elmadfa, 2009; Abdul-Razzak et al., 2011; Khuri-Bulos et al., 2014). Khatib & Elmadfa (2009) reported the high prevalence of anemia, vitamin A and vitamin E deficiencies of Bedouin School children in Northern Badia of Jordan as a consequence of poverty and undernutrition. They recommended multicomponent interventions for such multifaceted nutritional problems. Abdul-Razzak et al. (2011) studied the prevalence of vitamin D deficiency among healthy infants and toddlers in Irbid, Jordan. They found a high prevalence of vitamin D deficiency among northern Jordanian infants and toddlers. They concluded that sun exposure of less than 30 min daily and exclusively breast-feeding could be the main factors for developing vitamin D deficiency.

Although some intervention trials were carried out on Jordanian children, no specific interventions were adapted to adults. The problem of vitamin deficiency existed in adult Jordanian, where several studies were conducted to investigate factors leading to vitamins and minerals deficiencies in adult Jordanians. Mishal (2001) studied the effects of dress styles on the vitamin D levels in Jordanian women. Dress styles covering the whole body, totally or nearly totally, were found to have adverse effects on vitamin D levels and may produce secondary hyperparathyroidism in the long run. Batieha et al. (2011) also investigated the effect of dress style and gender on vitamin D levels in Jordan. They concluded that higher prevalence of low vitamin D status in females in comparison with males, in addition to a higher prevalence in women wearing Hijab or Niqab in comparison with Western styles. They recommended the development of strategies to reduce the risk among women, particularly those wearing dress styles that cover most or all of their skin. Mallah et al. (2011) studied the status of vitamin D among Jordanians. Their findings emphasized the importance of vitamin D supplementation, especially among conservatively dressed females.

Several studies indicated a trend of a low level of serum vitamin B_{12} in Jordanian adults (Hakooz et al., 2006; Barghouti et al., 2009; Qutob et al., 2011; El-Khateeb et al., 2014; El-Khateeb et al., 2019; Abu-Shanab et al., 2021). According to the researchers, the high frequency of low vitamin B_{12} deserves the development of a strategy to correct this problem in Jordan.

A high prevalence (37.3%) of recurrent aphthous stomatitis was reported in Jordanian patients suffering from hematinic deficiencies (vitamin B_{12}, folic acid, and iron) in addition to zinc. Treatment of hematinic deficiencies with proper nutrients such as B_{12}, iron and zinc could help in the management of the disease (Souza et al., 2017). Among many factors, low dairy products intake could be a risk factors for the low levels of vitamin B_{12} among the Jordanian population (Abu-Shanab et al., 2021).

It was reported that people in Jordan don't take multivitamins and minerals routinely. The overall prevalence rate of vitamin-mineral supplement use was very low and the main reason for supplement use was treatment (Suleiman et al., 2008; Ibrahim, 2018).

In conclusion, due to the findings of low blood levels of several vitamins and minerals in Jordanians, a proper strategy of intervention should be adopted by the country leaders. The intervention direction toward multivitamin tablets could be a bad choice since adult Jordanians don't like to take multivitamin tablets (Suleiman et al., 2008) and regarding multivitamin syrups, several products suffer from the unpleasant taste and many children refused their ingestion.

The objective of this study is to use widely accepted foodstuff as a medium to carry the deficient vitamins (D_3 and B_{12}) and minerals (iron, and zinc) as an acceptable tool for the Jordanian people. Dark chocolate is considered one of the preferred nutritious and healthy foodstuffs (Samanta et al., 2022).

Chocolate fortification could be widely accepted by both children and adults especially when the proper chocolate formulation is used. Such preparation should not change the taste of chocolate upon the addition of such vitamins and minerals, and also it should not significantly change the rheological, mechanical and textural properties of chocolate (Samanta et al., 2022). This study tried to examine if the addition of such micronutrients to chocolate could result in suitable chocolate in terms of content uniformity, taste, texture, mechanical, rheological and viscoelastic behavior.

2 Materials and methods

2.1 Materials

Sugar-free dark chocolate mass or suspension and maltitol (a sugar alcohol/substitute) were obtained from Al-Zaytuna for Chocolate & Confectionery Manufacturing Company (TOPS®), Amman, Jordan. Vitamin D_3, vitamin B_{12}, zinc sulfate, and ferrous sulfate were obtained from Dar Al Dawa Veterinary & Agriculture Industry Company (DAD vet), Amman, Jordan.

2.2 Preparation of fortified chocolates

Forty kg of the sugar-free dark chocolate mixture was used to prepare sugar-free dark chocolate bars enriched with minerals and vitamins.

The sugar-free dark chocolate mass was melted in a temperature-controlled jacketed container (temperature was kept at 55 ± 5 °C) and mixed using a shaft mixer. Stainless steel balls were placed in the bottom of the container to attain a homogeneous suspension. Vitamins and minerals were added according to (Table 1). The vitamins and minerals were geometrically mixed with a 0.5 kg

Table 1. Summary of content of micronutrients added to 10 kg batch of chocolate.

	Amount (mg) added to 10 kg	The amount in 60-g chocolate bar (mg)	RDA for added vitamins and minerals
Vitamin D3	4.17	0.015 (600 IU)[a]	15-20 mcg (600-800 IU)
Vitamin B12	0.4	0.0024	2.4-2.8 mcg[b]
Elemental Fec	1333	8	8 -27 mg[d]
Elemental Zn	1333	8	8 -13 mg[e]

[a]Each 25 microgram of vitamin D_3 is equivalent to 1000 IU. Vitamin D_3 was added to one of the chocolate products; [b]Recommended Dietary Allowance (RDA) for vitamin B_{12} depends on the age of user 14 years and older. Vitamin B_{12} was added alone to one of the chocolate products; [c]Zinc and iron were obtained from zinc sulfate and iron sulfate, respectively. Iron and zinc were added together to one of the chocolate products; [d]RDA for Fe depends on the age of user of 9 years and older, RDA is 8 -27 mg; [e]RDA for Zn depends on the age of user of 9 years and older, RDA is 8 -13 mg.

chocolate suspension fraction before the addition to the bulk chocolate suspension present in the container. Mixing was continued for 1 h to achieve homogeneity of the vitamins and minerals within the chocolate suspension. The hot chocolate suspensions were poured into molds to produce 60 g bars and let cool. The bars were hermetically sealed with food-safe aluminum foils and kept at room temperature until their use.

2.3 Characterization of the fortified chocolate bars

Chocolate bars fortified with minerals and vitamins were evaluated in terms of chemical composition using FTIR (Fourier transform infrared) and DSC (differential scanning calorimetry) methods, visco-mechanical evaluation (temperature/viscosity, softening point) and sensory evaluation (taste studies).

FTIR studies

The IR spectra of the sugar-free dark chocolate control (without the addition of micronutrients) and fortified chocolate bars with micronutrients were measured using a Fourier transform infrared (FTIR) spectrophotometer (Spectrum One, Perkin-Elmer, Buckinghamshine, UK) using KBr discs. A small sample (10 mg) was pulverized, gently triturated with the 200 mg KBr powder and then compressed using a hydraulic press at a force of 10 t. The disc was then placed in the sample holder and scanned from 4000 to 400 cm^{-1}.

DSC studies

Differential scanning calorimetry (DSC) was used to characterize the thermal properties of the chocolate upon the addition of micronutrients using a Mettler Toledo DSC823e DSC (Mettler Toledo, Switzerland). Around 5 mg samples were heated over a temperature range from 25 to 300 ˚C at a rate of 10 ˚C/min in sealed aluminum pans.

Softening point test

The softening point test was performed on the fortified chocolate compared to plain chocolate (Lucena et al., 2004). The test was conducted based on the American Society for Testing and Materials, ASTM Designation D36-95 according to the Method for Softening Point of Bitumen (Ring-and-Ball Apparatus) with a heating rate of 5 ˚C/min.

Viscosity measurements

The viscosity was determined for the fortified chocolate and the results were compared with plain chocolate using a viscometer (Rotational Viscometer RV, V1-L, TQC B.V., The Netherlands) at different temperatures and rotational speeds. Samples were heated in a water bath for 1 h with mixing at 75 ˚C. The samples were placed in the viscometer chamber and let to cool to room temperature and the viscosity was taken at temperatures 50, 60, and 70 ˚C (First run melting). The samples were let to cool to room temperature and heated again to 75 ˚C and the same procedure of viscosity measurement was repeated (Second run melting).

Taste evaluation

This is an important test to evaluate an important parameter that could significantly affect the taste of the chocolate by the addition of vitamins and minerals. All the taste panel signed written informed consent. Exclusion criteria of the taste panel: smokers (> 10 cigarettes/day), a person having any type of inflammations in the mouth and unhealthy volunteers (diabetes, asthma, allergic patient).

The Procedure of Sensory Sensation (Taste Evaluation):

First: taste one cube (~6 g) of chocolate (control) and melt it in the mouth slowly, then after 1 minute of complete absorption, evaluate the taste. Second: wash the mouth with 100 mL of water. Third: for each fortified chocolate taste one cube of chocolate (~6 g) and wait till complete melting after 1 min record the taste relative to control and wash mouth with water between each trial. A summary of parameters used to evaluate the taste of chocolate is listed in (Table 2).

2.4 Inverted microscope

Photos under an optical microscope (Primovert, Zeiss with axiocam ERc5s software MBT2011 configuration Crl Zeiss Microscopy GmbH Gottingen, Germany) for a sample of chocolate heated using a hot air shower where a thermometer was placed near the sample to monitor the temperature of the flushed hot air and at the same time to observe melting changes of the chocolate sample under the microscope.

2.5 The actual amounts of added minerals and vitamins and method of analysis

The actual amounts of the added minerals and vitamins are presented in (Table 3).

2.6 Extraction method of the minerals and vitamins from the chocolate

For iron, zinc and B_{12} extraction: weight about 10 g of chocolate and crush it into small pieces. Dissolve the crushed chocolate in 40 mL n-Hexane. Vortex/Shake for 5 min, then

Table 2. Sensory evaluation of chocolate and scoring.

Degree.	Sweetness	Flavor intensity	Texture after melt	Hedonic	Score (%)
1	very bitter	very faint	very hard	not tasty	25
2	bitter	faint	hard	slight tasty	50
3	slightly sweet	slightly intense	liquid creamy	acceptable taste	75
4	sweet	acceptable intensity	creamy	highly acceptable taste	100

Table 3. The actual micronutrient added amounts.

	Amount of minerals and vitamins per 10 g chocolate			
	Iron Sulfate	Zinc Sulfate	B12	D3
Actual amount	4.44 g	2.40 g	0.17 mg	4.17 mg
Assay	30%	35%	100%	100%
Mineral or vitamin amount/g Chocolate	133.2 µg Iron	120 µg Zinc	17 ng B_{12}	417 ng D_3

add 40 mL of water. Vortex/Shake the obtained dispersion for 5 min. Take the water layer (lower) and measure the iron content. For the extraction method of Vitamin-D_3: weight about 10 g of chocolate and crush it into small pieces. Dissolve the crushed chocolate in 20 mL THF and vigorous shaking/vortex for 5 min continuously. Add 50 mL water, and vigorous shaking by hand for 5 min continuously. Centrifuge for 2 min at 4400 rpm then inject. All results of Zn and iron were measured by medical lab kits using "Cobas C 111" instrument and "Cobas e 411" instrument for B_{12}. All results were measured by the API-4000 LCMSMS instrument.

2.7 Stability studies

A stability study under different temperatures (25, 40 and 75 °C) was carried out for the control and the fortified chocolate for nine months. Samples were withdrawn at different time intervals and analyzed.

3 Results and discussion

3.1 Preparation of fortified chocolates

The fortified chocolates containing the micronutrients were prepared as shown in (Table 1). The chocolate bars were characterized in terms of physicochemical, mechanical, stability and taste properties. The use of maltitol in chocolate production is important because it makes chocolates "tooth-friendly" and "sugar-free." Maltitol is a sugar substitute that is added to sugar-free dark chocolate as a sweetening agent; it is a substitute that has 75-90% of the sweetness of sucrose and its melting point is 145 °C (Saraiva et al., 2020). The average concentration (per g chocolate) of natural iron, zinc, B_{12} and D_3 in the control samples were 5.5 µg, 11 µg, 10 ng, almost zero, respectively. The added amounts of the micronutrients indicate that the chocolate has been successfully fortified with the micronutrients as compared to control samples.

3.2 FTIR studies

Fourier transform infrared (FTIR) spectroscopy is an appealing technology for the food industry because simple, rapid, and nondestructive measurements of chemical and physical components can be obtained. Advances in FTIR instrumentation combined with the development of powerful multivariate data analysis methods make this technology ideal for large volume, rapid screening and characterization of minor food components down to parts per billion (ppb) levels. FTIR has been used as a useful tool for identification and also detecting adulteration of chocolate (Man et al., 2005; Didar, 2021). In this study, FTIR was used to detect the changes in chocolate FTIR spectra after the addition of the micronutrient compared to the control (plain chocolate). The most characteristic spectral band are 1743 cm-1 stretching vibration of carbonyl groups (C=O) from esters of triglycerides, 3007 cm-1 stretching vibration cis C=C, 2691 cm-1 asymmetric stretching vibration of methyl groups ($-CH_3$), 2921 cm-1 symmetric stretching vibration of methylene groups ($-CH_2-$), 1458 cm-1 bending vibration of CH_2 and CH_3 aliphatic groups C-O and 1234 cm-1 stretching vibration in ester. The spectrum of all fortified chocolates was similar to that of plain chocolate and there were no peaks for micronutrients. This could be attributed to the very low concentration of micronutrients and at the same time the presence of a high concentration of several components in chocolate.

3.3 DSC studies

A summary of the most characteristic peaks of the DSC thermogram of the different chocolate preparations containing micronutrients (vitamin B_{12}, vitamin D_3, zinc and iron) compared to control (plain chocolate) is given in (Table 4).

All the samples exhibited similar distinct single endothermic transitions between 15 °C and 55 °C, the range expected for dark chocolate melting profiles. The thermogram showed similar peak shapes and sizes for dark chocolates manufactured with varying amounts of micronutrients, suggesting no characteristics differences in crystallinity and degree of crystallization between the fortified products.

Micronutrients usually possess high melting points (MP): zinc sulfate, anhydrous (680 °C) (Hammond, 2005), iron sulfate, anhydrous (680 °C) (Lide, 2009), and vitamin B_{12} (MP > 300 °C) (American Chemical Society, 2019). The lowest MP among all micronutrients was for vitamin D_3 (MP 84-85 °C) (O'Neil, 2001). These micronutrients with higher melting points than chocolate components may serve as crystal promoters that affect crystallization during the cooling of the chocolate compounds. The cocoa butter base melting point decreased from 38.3 to 33.5 °C upon the addition of such micronutrients (Rosales et al., 2017).

3.4 Softening point

The softening point of the Bitumen test was carried through a special assembly where balls are placed on top of a layer of the chocolate present in the ring (mold). The temperature is raised gradually and the temperature at which the balls hit the bottom of the container is recorded as the softening point.

Table 4. Summary of the most characteristic peaks of the DSC thermogram.

Item	Endo. Peak 1 (°C)	Endo. Peak 2 (°C)	Exo. Peak (°C)
Control	38.3	142	284
Zn + Fe	33.5	144	> 300
D3	33.5	144	285
B12	33.5	144	> 300

The softening point of the 3 types of the fortified chocolates was 35 ˚C and that of the plain chocolate (control) was 32 ˚C. Although micronutrients are present in minute amounts, it seems that they were able to strengthen the structure of the chocolate base which could result in a slight shift in the melting point of both cocoa butter base and maltitol sugar present in the chocolate composition. This also suggests that such micronutrients were mixed evenly within the structure of the chocolate base and were compatible with chocolate components. The decomposition temperature (exothermic peak) for the fortified chocolate was increased above 300 ˚C compared to 284 ˚C for the plain chocolate except for D_3 (285 ˚C) which may be due to the low melting point of this component compared to other micronutrients. While crystal promoters added to compound chocolate affect their viscosity and thermal behavior (Rosales et al., 2017). Another proof of increasing the chocolate strength was by increasing the time required for penetration of the stainless-steel balls through chocolate.

3.5 Rheology studies

Generally, chocolate formulations are mixtures containing solid particles made from a combination of milk powder, cocoa powder and sugar. The successful ability to produce a homogeneous liquid fat phase from this mixture and produce a good quality final product depends in part on the Rheology of the formulation. Rheology is the science that is concerned with the flow properties and flow parameters such as yield stress, viscosity vs. shear rate and viscosity vs. temperature. All the tested molten chocolate samples (control and fortified samples) were found to be pseudoplastic (shear-thinning). The results of chocolate viscosity test data at a shear rate vary from 0.833 to 3.33 s^{-1} (50 to 200 rpm). The effects of three temperatures (50, 60 and 70 ˚C on the rheological properties of the chocolates were examined. For control and tested samples, viscosity decreased with increased shear rate. For control samples, viscosity decreases from almost 9000 mPa.s (at 50 ºC), 1100 mPa.s (at 60 ºC) and 14300 mPa.s (at 70 ºC) to under 5000 mPa.s as the shear rate increases. Decreasing the shear rate thereafter shows that the viscosity does not recover right away. There is some time sensitivity to the shearing action which results in a temporary loss of structure in the chocolate. Generally, chocolate fortification doesn't cause significant variation in the rheological properties of chocolate samples.

3.6 Inverted microscope

Images for the chocolate at room temperature (25 ˚C) showed a dark field background indicating a non-molten mass. As the temperature increases, the area of the dark field decreased indicating an increased ratio of the molten mass.

3.7 Sensory evaluation

Data of the individual results of the different sensory parameters indicated that fortification of the chocolate with the micronutrients has no significant ($p > 0.005$) effect on the overall sensation of taste when compared with controlled chocolate.

3.8 Stability study

The stability results indicated that there is no significant ($p > 0.005$) decrease in the assay of the minerals and vitamins for 9 months at room temperature**.**

4 Conclusion

It was concluded from this study that the micronutrient-fortified chocolate was a good vehicle for the vitamins and minerals with acceptable physical, chemical, and sensory properties that may be used as a delicious food for supplementing vitamins and minerals. Practically, this type of fortified dark chocolate may be one of the available and affordable food products that help to combat vitamins and minerals deficiencies in Jordan as well as other countries. It is also a suitable confectionery and snack for diabetics and for weight-reducing regimen because it is sugar-free, and a healthy food choice fortified with the most deficient vitamins and minerals.

Acknowledgements

This study was basically supported by the Higher Scientific Council of Jordan/Industrial Research and Development Fund (project no. 713/18/1) and got a partial financial support from the Deanship of Scientific Research at the University of Petra under project number (10/4/2018). Authors acknowledge the support of Al Zaytuna Company manager, Eng. Saleem Hundeilah, for allowing the conduction of the trials in the facility of the company.

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