

# Production of Infant Formula Using Mixture of Walnut and Almond Oil

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**Abstract:** Milk is an essential food for children and it is clear that the best source of nutrition for babies is breast milk. The World Health Organization (WHO) recommends that infants be exclusively breastfed for the first six months of life. However, some babies may need complementary milk during the early months of life or may be weaned for some reason. Although it is not possible to produce the same product as breast milk, attempts have been made to imitate the nutritional characteristics of breast milk for the normal growth and development of infants. Infant Formula is considered as an effective alternative to breast milk and has been prepared to mimic the nutritional composition of breast milk. Formulas need to meet the factors of normal physical growth quality and adequate biological quality. The production process is also fully regulated and monitored to meet national and international quality standards. Today, cow's milk is the most widely used alternative to human milk and the basis of many Infant Formulas. In this study, the effect of adding walnut and almond oil to infant formula was investigated. The number of treatments was determined by using the Mixture Design method and the necessary tests were performed for infant formula and physicochemical and qualitative tests including moisture, dry matter, measurement of color parameters, density, particle size, insoluble index, pH, acidity, total sugar, fat, protein, ash, vitamin C, minerals, aflatoxin M1, wettability acceptable and scorched particles were done. The results showed that adding this formula could meet 90% of our expectations for the production of infant formula and not have an adverse effect on the physicochemical properties of the formula.

**Keywords:** Infant Formula, Walnut Oil, Almond Oil

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## 1. Introduction

Most milk formulas are formulated to resemble the nutritional composition of breast milk [1].

Generally, the essential ingredients of infant formula include carbohydrate, protein, fat as well as vitamins and minerals to meet the needs of a growing full-term infant. Other important components, which should be present, include choline, inositol and long chain polyunsaturated fatty acids [2, 3].

The blends of vegetable oils used in infant formulas are selected to match the excellent absorption by the infant of breast milk fat, but aside from the absence of long-chain

polyunsaturated fatty acids (LCPUFA), they differ considerably from human milk fat in their fatty acid profiles [4]. Palmitic acid is the major saturated fatty acid in breast milk, accounting for 17–25% of the total [5, 6]. Palm oil and its low melting fraction, palm olein, a relatively inexpensive source of palmitic acid, are added to many infant formulas in amounts that mimic the palmitic acid content of human milk [7, 8].

Most fatty acids are better absorbed as monoglycerides than as free acids because monoglycerides form mixed micellae with bile acids and cannot form complexes with divalent cations. Fatty acids in the sn-2 position are absorbed as soluble 2-monoacylglycerides, while those in

the sn-1 and sn-3 positions are absorbed as free fatty acids [9]. Most infant formulas use vegetable oils in place of milk fat to provide an overall fatty acid profile similar to that of breast milk. Vegetable oils have 5 to 20% saturated fatty acids in the sn-2 position of triglycerides unless they are modified by interesterification [10]. Interesterification is increasingly used for the fat for infant formulas to raise the level of saturated fatty acids in the sn-2 position to 40 to 60% [11]. The role of a plant-based diet on a decrease in cardiovascular diseases, Pistollato *et al.* (2018) observe that a diet rich in plant-based foods including soybeans and nuts reduces the risk of neurodegenerative disorders such as Alzheimer's disease. Moreover, Rita and Luciana (2018) state that even a daily consumption of only two glasses of cow's milk results in an intake of D-galactose above 100 mg/kg which could cause Parkinson's disease [12, 13]. In this context, oilseed and nut milk substitutes—almond (*Prunus dulcis*), cashew (*Anacardium occidentale*), coconut (*Cocos nucifera*), hazelnut (*Corylus*), peanut (*Arachis hypogaea*), sesame (*Sesamum indicum*), soy (*Glycine max*), tiger nut (*Cyperus esculentus*), oat (*Avena sativa*), rice (*Oryza sativa*), hemp (*Cannabis sativa*), and walnut (*Juglans*)—are preferred by vegans and people who suffer from an allergy to cow's milk [14, 15]. The health effects of plant-based milk substitutes have been studied in terms of both positive and negative effects. Plant-based milk substitutes have positive effects because of rich antioxidant activity and fatty acid which reduce the risk of cardiovascular diseases, cancer, atherosclerosis, and diabetes. However, plant-based milk substitute products also have various negative health effects including lack of protein content, low bioavailability of minerals and vitamins, and oral health problems. One problem, the low bioavailability of vitamins and minerals because of some anti-nutrients and polyphenols, can be overcome by fermentation. In this study, the effect of adding walnut and almond oil to infant formula was investigated [16, 17].

## 2. Materials and Methods

### 2.1. Sample Preparation

Fresh cow's milk was collected from cow farm in shahrekord province, Iran. Mean fat, protein, lactose, aflatoxin M1 in whole cow's milk were measured (Table 1).

### 2.2. Pasteurizing Milk

Milk samples were pasteurized at 72°C for 15 second using a pasteurizer manufactured by Niksanat Iran and transferred to a cold room at 4°C for cooling [18].

### 2.3. Separation of Cream

Whole milk cream (milk at 4°C) was separated at 3500 g for 10 minutes using a 2-16P desktop centrifuge made by the German company Sigma, with a capacity of 50 ml per cup. The separated cream was removed using a spatula [19].

### 2.4. Producing Infant Formula

Almond and walnut kernels were purchased from Iranian Local Market. To prepare the emulsion, demineralized whey powder, lactose powder, vegetable oil and other components of infant formula were added to cow and milk using a magnetic stirrer. The mixture was heated to 50°C by Ben Marie and homogenized for 2 minutes using an ULTRA-TURRAX T25 basic homogenizer made by the German company IKA-WERKE at a speed of 11000 rpm for 2 minutes. Infant formula was produced using spray drying method. In this research, a spray dryer brand BUCHI model b-290 made in Switzerland, which was equipped with a liquid nozzle with a water evaporation capacity of 1 liter per hour, was used. The atomic compressor air intake was set at 40 kPa. The inlet and outlet temperatures of the drying air were controlled at 160°C and 70°C, respectively. The powders were collected and placed in vacuum sealed aluminum bags [20, 21].

## 3. Determination of Physicochemical Composition

### 3.1. Moisture and Dry Matter & Protein & Fat & Ash & Carbohydrate

Moisture content of infant formula powders was measured by AOAC method with the help of an oven (model U630 made by Fater Electronic Company of Iran) 1 gram of sample was dried at 105°C for 4 hours (105±1°C for 4 h) Or until a constant weight difference of 0.001 g was obtained. Protein content was measured using the AOAC method and with a Kjeldahl digester (TR model of Gerhardt company in Germany). The amount of fat content was calculated using AOAC method and with Gerber centrifuge (NOVA model Gerber centrifuge made by the company (Funk Gerber). To measure the amount of ash using AOAC method, model AAF11/3 furnace made by Carbolite Company of America at 500°C was used. This process continued until white ash was obtained [22, 23]. The ash percentage was obtained by Equation (1).

$$\% \text{Ash} = \frac{W_A}{W} \times 100 \quad (1)$$

$W_A$ : Ash weight obtained

$W$ : Sample weight

To determine the amount of total Carbohydrate, the following equation was used according to the AOAC method:

Carbohydrate = total solids – (proteins + fat + ash)

### 3.2. Particle Size

To distribute the particle size, the powder samples were sieved through a number of JEL 200 sieves from Engelsmann, Germany, along with rubber blocks of different mesh sizes, using a horizontal oscillation motion and divided into sections with different particles size [24].

### 3.3. pH Measurement & Acidity

The pH of the samples was measured by the pH meter of the 766 Calimatic model made by the German company Knick, after reaching the temperature of LIF (liquid infant formula) at a temperature of 20 degrees Celsius. The titratable acidity was expressed as lactic acid and by titration a certain amount of milk reconstituted with 0.1 N NaOH was determined using phenolphthalein as an indicator [25].

### 3.4. Vitamin C

Because of vitamin C sensitiveness to heat, oxygen and light, it was considered an indicator of vitamins. To measure the amount of vitamin C, the titration method (calibration method with reagents 2 and 6 dichlorophenolindophenol) was used according to Equation (2) (Source: [26]).

$$\text{vit c} \frac{\text{mg}}{100\text{g}} = \frac{V \times V_m \times 100 \times F}{V_s \times W} \quad (2)$$

V=usage of volume dichlorophenolindophenol solution

V<sub>m</sub>=volume of Sample and metaphosphoric acid solution

F=mg of ascorbic acid equivalent to 1 ml of dichlorophenolindophenol solution

V<sub>s</sub>=the volume of filtered solution

W=the initial weight of the powder sample

### 3.5. Phosphorus & Metals Measurement

To measure phosphorus, after preparing the sample (digestion and creating a color complex), the sample was absorbed using a UV mini-1240 spectrophotometer from Shimadzu, Japan, equipped with a 10 mm long tube measuring 820 nm. To measure the number of metals including iron, calcium, magnesium, potassium, sodium, zinc and copper, the sample was first thoroughly dried by a furnace and then the remaining ash was dissolved in dilute nitric acid. The dissolved solution was injected into the XS104 atomic absorption spectroscopy device of the American company Thermo and a specific wavelength and a special lamp for the metal were used for each metal [27].

### 3.6. Insoluble Index & Scorched Particles & Wettability & Bulk Density

The measurement of the insoluble index is the ability of the powder to dissolve in water and as a means of assessing solubility. To determine it, the powder was dissolved in water at a certain temperature. The volume of sediments (insoluble residue) after centrifugation of the sample was reported in milliliters by Gerber Univ model centrifuge made in Germany. The number of particles scorched in the powder using GEA Niro Method No. A 4 a, and then a comparison with the ADMI chart (Tables 2 and 3) were determined. To obtain the ability to wet the powder samples, a certain amount of powder was poured into water at a certain temperature, according to the Niro method used for milk powder and other dry dairy products. If the powder does not get wet after 5 minutes, the analysis stops and the result is > 300 seconds. To measure the

bulk density, a Stampfvolumeter device made by the German company Engelsmann was used [28]. The powder sample was transferred to a measuring cylinder and 100 beats were applied to it and calculated according to Equation 3 in kg / m<sup>3</sup>.

$$\rho_{100} = \frac{m}{v_{100}} \quad (3)$$

### 3.7. Aflatoxin M1

Aflatoxin M1 was analyzed by liquid chromatography (HPLC). To measure aflatoxin M1, C18 column and fluorescent detector at 30°C with emission of 405 nm with reverse phase chromatography of Hitachi model made in Japan were used. The injection volume of the solvents used was 50 ml of 40% sodium and methanol buffer. All chromatographic data were reprocessed and analyzed in ELSI software [29].

### 3.8. Colorimetric Test

Colorflex EZ colorimeter (Hunterlab, USA) was used to determine the color status of powder samples. Powder samples were placed in the cup of the device with a diameter of 90 mm and a thickness of 20 mm at a temperature of 25°C. In the CIELAB system, the factors were L\* (dark and light), a\* (red and green) and b\* (yellow and blue) were measured. All measurements were performed in triplicate for each sample. The range of L values ranged from 0 (white) to 100 (black) [30].

### 3.9. Particle Microstructure

Particle's morphology was analyzed by scanning electron microscopy (SEM) using a microscope with X-ray dispersive energy detector (Leo 440i EDS 6070, Leica Elctron Microscopy, England). An acceleration voltage of 15 kV and a beam current of 50 pA were used. Initially, samples were subjected to metallic coating using the Sputter Coater POLARON (SC7620 model, VG Microtech, England). Images (with 6,000x and 10,000x magnification) were captured using the software LEO v. 3.01 [31].

### 3.10. Statistical Analysis

All experiments were carried out using three replicates and the data are presented as the mean values±standard error of the mean (SE). For statistical analysis we either used one-way analysis of variance (ANOVA) in design expert13.

## 4. Results and Discussion

The number of treatments was determined by using the Mixture Design method and the necessary tests were performed for infant formula and physicochemical and qualitative tests including moisture, dry matter, measurement of color parameters, density, particle size, insoluble index, pH, acidity, total sugar, fat, protein, ash, vitamin C, minerals, aflatoxin M1, wettability acceptable and scorched particles were done.

**Table 1.** Results of statistical analysis of the studied parameters and proposed equations by Mixer Design method in Design Expert software.

Parameter	A Walnut	B Almond	AB (Walnut Almond)	AB (A-B)	AB (A-B) <sup>2</sup>	F-value	R-Squared	Model	s/Ns
Fat	+0.29	+0.27	$10^{-4} \times -3.84$	$10^{-6} \times +6.00$	-	47.08	0.97	Cubic	S**
Carbohydrate	+0.61	+0.62	-	-	-	24.59	0.80	Linear	S**
Ash	+0.02	+0.02	-	-	-	9.14	0.60	Linear	S*
Vitamin c	+0.47	+0.39	$10^{-3} \times -2.47$	-	-	17.54	0.88	Quadratic	S**
Protein	+0.11	+0.12	-	-	-	26.58	0.82	Linear	S**
moisture	+0.018	+0.014	$10^{-5} \times +3.88$	$10^{-6} \times -2.57$	-	12.07	0.90	Cubic	S*
dry matter	+0.98	+0.99	$10^{-5} \times -3.76$	$10^{-6} \times +2.57$	-	12.54	0.90	Cubic	S*
pH	+0.068	+0.067	$10^{-5} \times +6.05$	$10^{-7} \times -9.47$	-	21.05	0.94	Cubic	S**
Acidity	-	-	-	-	-	-	0.64	Cubic	Ns
Phosphorus	+0.49	+0.47	$10^{-4} \times +2.25$	$10^{-5} \times -1.01$	-	-	0.99	Cubic	S**
Iron	+9.46	+0.01	$10^{-5} \times +2.31$	$10^{-6} \times -1.46$	-	11.34	0.90	Cubic	S*
Calcium	+0.76	+0.68	-	-	-	10.51	0.64	Linear	S*
Copper	+0.67	+0.66	$10^{-3} \times -1.38$	-	-	63.40	0.96	Quadratic	S**
Potassium	+1.30	+1.17	-	-	-	12.31	0.67	Linear	S*
Zinc	-	-	-	-	-	-	0.01	Linear	Ns
Magnesium	+0.08	+0.10	-	-	-	77.12	0.93	Linear	S**
Sodium	+0.31	+0.27	$10^{-4} \times -3.65$	-	-	168.74	0.99	Quadratic	S**
L *	-	-	-	-	-	-	0.58	Cubic	Ns
a *	-0.04	-0.05	$10^{-4} \times +1.19$	$10^{-6} \times -1.53$	$10^{-8} \times +4.57$	15.13	0.95	Quartic	S*
b *	+0.12	+0.14	$10^{-4} \times -5.25$	$10^{-6} \times +9.65$	-	23.93	0.95	Cubic	S**
EΔ	+0.09	+0.09	$10^{-5} \times -5.58$	$10^{-7} \times -5.42$	$10^{-8} \times -2.46$	17.84	0.96	Quartic	S*
Aflatoxin	-	-	-	-	-	-	0	Mean	Ns
Particle size	-	-	-	-	-	-	0	Mean	Ns
Density	-	-	-	-	-	-	0	Mean	Ns
Insoluble Index	-	-	-	-	-	-	0.44	Quadratic	Ns
Wettability	-	-	-	-	-	-	0	Mean	Ns

S\*: significance at 5% level

S\*\*: significance at 1% level

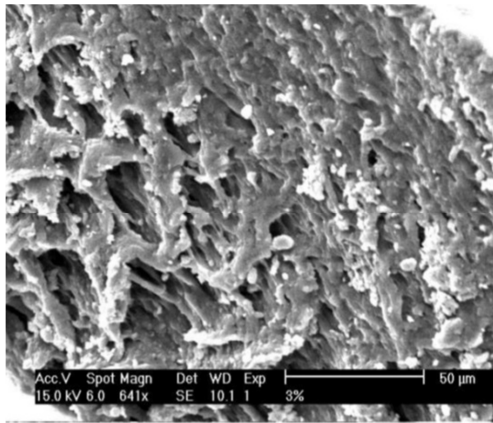
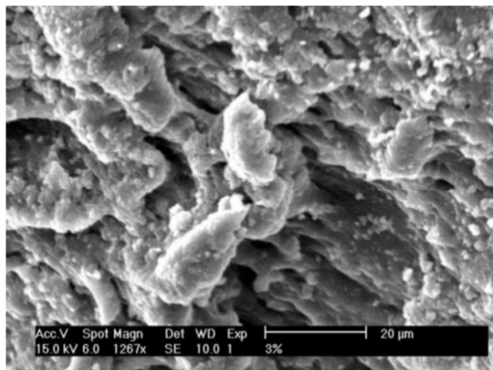
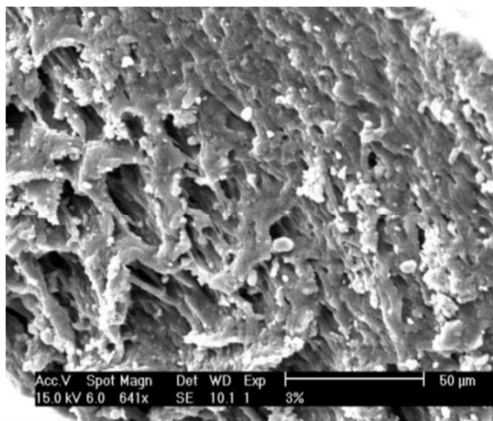
Ns: non-significant.

**Table 2.** Optimizing the mixing of infant formula.

Parameter	unit	Standard acceptance range		The optimal value obtained by the software	Experimental value	Reference
		Min	Max			
Fat	%	22.53	30.72	26.40	26.10±1.04	Codex Alimentarius
carbohydrate	%	46.08	71.68	61.69	60.71±2.14	Codex Alimentarius
Ash	%	0	3	2.12	2.09±0.11	Commission on Dry Milk
Vitamin c	mg/100kcal	10	50	36.50	36.25±0.82	Commission on Dry Milk
Protein	%	9.22	15.36	11.35	11.22±0.27	Codex Alimentarius
Humidity	%	0	3	1.74	1.72±0.05	Codex Standard
dry matter	%	97	100	98.26	98.30±0.05	Codex Standard
Acidity	g/100ml Lactic acid	0	0.14	0.04	0.04±0.010	Codex Standard
Phosphorus	mg/100kcal	25	100	48.68	48.50±0.32	Codex Alimentarius
Iron	mg/100kcal	0.5	2	1.2	1.20±0.03	Codex Standard
Calcium	mg/100kcal	50	140	70.84	70.80±0.13	Codex Alimentarius
Copper	μg/100kcal	35	120	62.98	61.56±0.50	Codex Alimentarius
potassium	mg/100kcal	60	180	122.21	119.11±3.88	Codex Alimentarius
Zinc	mg/100kcal	0.5	1.5	0.65	0.64±0.21	Codex Alimentarius
Magnesium	mg/100kcal	5	15	9.08	9.03±0.21	Codex Alimentarius
Sodium	mg/100kcal	20	60	27.5	27.43±0.11	Codex Alimentarius
L *	-			92.95	92.88±0.10	-
a *	-			-3.94	-3.99±0.08	-
b *	-			11.59	12±0.08	-
ΔE	-			8.87	8.93±0.11	-
Aflatoxin	Ppt	0	25	10.17	10.09±0.05	Food & Feed Mycotoxins
Particle size	Micro meter			Ns	-	-
Density	kg/m3			445.63	445.42±0.31	-
Insoluble Index	Ml			0.10	0.10±00	-
Wettability	Minute			Ns	-	-

**Table 3.** Nutrients added to formula.

Parameter	unit	demineralized whey powder	Lactose	Oil	Premix Vitamin	Premix Minerals
Protein	%	5.44	-	-	-	-
Fat	%	-	-	27	-	-
carbohydrate	%	37.23	12.07	-	-	-
Vitamin c	mg/100kcal	-	-	-	57.29	-
Iron	mg/100kcal	-	-	-	-	0.8
Sodium	mg/100kcal	7.70	-	-	4.49	1.34
Potassium	mg/100kcal	17.11	-	-	-	41.03
Phosphorus	mg/100kcal	10.28	-	-	-	1.04
Copper	µg/100kcal	-	-	-	-	61.88
Zinc	mg/100kcal	-	-	-	-	0.50
Magnesium	mg/100kcal	2.14	-	-	-	2.44
Calcium	mg/100kcal	17.13	-	-	-	9.58

**Figure 1.** SEM for almond oil.**Figure 2.** SEM for walnut oil.**Figure 3.** SEM for almond and walnut oil.

## 5. Conclusions

The use of powdered formula that is free of palm olein and palm kernel oil is associated with improved intestinal absorption of the major fatty acids and total fat, as well as calcium retention. The results showed that adding this formula could meet 90% of our expectations for the production of infant formula and not have an adverse effect on the physicochemical properties of the formula.

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