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Preliminary communication

EFFECT OF THE TEMPERATURE AND pH ON METHANOL RELEASE IN COFFEE BREW SWEETENED WITH ASPARTAME

G.V. PORTARI[a]*, M.G.M. MATHIAS[a], B.B. ALMEIDA[a], J.S. MARCHINI[b] and A.A. JORDAO[a]

[a] Course of Nutrition and Metabolism, Faculty of Medicine of Ribeirao Preto – University of Sao Paulo. Av Bandeirantes 3900. Ribeirao Preto/SP. Brazil. 14049-900
[b] Department of Internal Medicine, Faculty of Medicine of Ribeirao Preto – University of Sao Paulo. Av Bandeirantes 3900. Ribeirao Preto/SP. Brazil. 14049-900

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Methanol is an alcohol which is metabolized to folmaldehyde in humans. It is a very toxic substance, responsible for blindness in cases of methanol intoxication. This study shows the production of methanol when aspartame is used to sweeten coffee brew. The temperature versus pH binomium was also tested. When assayed at 90 °C coffee brew+aspartame and aspartame solution showed an increase in methanol release of 17.8 and 19.3%, respectively, as compared with coffee brew. At 180 °C, the increase was 32.5 and 26.3%, respectively. Our data revealed a protective effect of the pH of coffee on the degradation of aspartame and formation of methanol; an important fact, mainly for specific populations that use aspartame, like diabetics.

Keywords: aspartame, coffee brew, methanol

The stability of aspartame, when dissolved in water, depends markedly on pH. At room temperature, it is most stable at pH 4.3, where its half-life is nearly 300 days. At pH 7, however, its half-life is only a few days (GRAVES & LUO, 1987; BELL & LABUZA, 1991; SKWIERCZYNSKI & CONNORS, 1993; PATTANAARGSON & SANCHAVANAKIT, 2000; CONCEIÇÃO et al., 2005).

After being ingested, aspartame is completely metabolized into its primary constituents, i.e. aspartic acid, phenylalanine and methanol. After absorption, methanol is carried to the liver through the portal circulation and is metabolized there to formaldehyde, a highly reactive and toxic substance which, among others, may cause blindness (DAVOLI et al., 1986; LEON et al., 1989; STEGINK et al., 1989; TROCHO et al.,

* To whom correspondence should be addressed.
Phone: +55-15-16-36024629; e-mail: gv_portari@yahoo.com

1998). TROCHO and co-workers (1998) reported the formation of adducts by the binding of formaldehyde produced by aspartame injection to proteins and DNA.

The public health implications of these findings may be substantial, since aspartame is used in about 6000 products, and more than 200 million people regularly consume aspartame through foods, beverages, drugs, and hygiene products such as toothpaste (FURDA et al., 1975). In Brazil, aspartame is the second most frequently used sweetener, being also used to sweeten coffee brew (TOLEDO & IOSHI, 1995).

Another mechanism by which methanol can be formed from aspartame is the lability of aspartame under certain conditions of temperature and pH (FURDA et al., 1975; BELL & LABUZA, 1994; SABAH & SCRIBA, 1998). Thus, at temperatures higher than 150 °C, complete degradation can occur in aqueous medium, whereas at alkaline pH decomposition occurs already at 30 °C.

Coffee is a drink extensively consumed the world over. Culturally, coffee is a drink consumed hot which is produced by infusion of the powder of roasted beans in boiling water and then sweetened. Because of the low stability of aspartame in aqueous systems and the high consumption of coffee by the population in general, the objective of the present study was to determine the production of methanol when aspartame is used to sweeten coffee. The temperature versus pH binomium was also tested.

1. Materials and methods

1.1. Materials

Roasted coffee and liquid aspartame (8% aspartame, w/v) obtained from the local commerce were used for sample preparation. The coffee samples were prepared by infusion with boiling water, in the proportion of 50 g of coffee powder to 200 ml of water followed by filtration through a commercial paper filter. The temperature of coffee after infusion/filtration was measured with a thermometer with 0 to 150 °C graduations and, after cooling to room temperature, the pH was measured with a pH-meter.

1.2. Test solutions

Five different solutions were prepared for determination of methanol: 1) Pure coffee (C), to determine the methanol naturally present in the coffee brew; 2) coffee plus liquid aspartame (CA) (10 drops in 10 ml of coffee); 3) an aqueous solution of aspartame (AS) (10 drops in 10 ml of deionized water); 4) an acidified aqueous solution of aspartame (AAS) (10 drops of aspartame in 10 ml of citrate buffer, pH 4.0); and 5) an alkalinized aqueous solution of aspartame (BAS) (10 drops in 10 ml of TRIS buffer, pH 8.5). The final concentration of the solutions that contained aspartame was 3.9 mg ml^{-1}.

1.3. Assays

The assays were carried out with 6 replicates. In each assay, 0.5 ml of the test solution was transferred to a 5 ml BD Vacutainer® blood collection tube containing 0.5 ml of

internal standard solution (acetonitrile) and sealed with a rubber stopper in order to prevent methanol escape. Each lot of tubes was then heated to 90 °C or 180 °C for 5 minutes.

1.4. Chromatographic conditions

Methanol analyses were performed by manual headspace high resolution gas chromatography using a Shimadzu GC-17A equipped with a DB-WAX (J&W Scientific) capillary column (l=30 m, I.D.=0.25 mm, dF=0.25 μm) (PORTARI et al., 2008). After cooling the samples to room temperature, the tubes containing the test solution were placed in a water bath at 60 °C for 20 min to equilibrate the headspace. A 150 μl aliquot of the headspace was injected into the gas chromatograph. The ratio of the area of the methanol peak to the area of the peak of the internal standard was used for quantification by means of a calibration curve for methanol at concentrations of 10, 51, 102, 1140 and 3081 μg ml^{-1}.

1.5. Statistical analysis

Data are reported as means±SD. The different lots were compared by the non-parametric Mann-Whitney test, with the level of significance set at $P<0.05$.

2. Results and discussion

After infusion, the temperature of the coffee brew was 90 °C and the pH was 5.

Table 1 presents the methanol values detected in pure coffee (C), in coffee plus aspartame (CA) and in the aqueous solution of aspartame (AS). Natural occurrence of methanol, regardless of the temperature used, was observed in the coffee brew. Methanol is naturally present in small amounts in coffee brews probably due to the hydrolytic effect of infusion with water on substances such as pectins since there was no change due to the effect of temperature.

Table 1. Quantity of methanol (μg ml^{-1}) released in pure coffee brew, coffee brew sweetened with aspartame and in the aspartame solution

Temperature (°C)	C	CA	AS	
90	$29.16\pm2.12^{a,1}$	$34.34\pm1.33^{b,1}$	$34.80\pm2.01^{b,1}$	P=0.0022
180	$38.28\pm3.11^{a,1}$	$50.70\pm3.93^{d,2}$	$48.33\pm3.92^{d,2}$	P=0.0095
	P=0.4848	P=0.0095	P=0.0043	

Different letters indicate statistical differences in the lines and different numbers indicate statistical differences in the columns ($P<0.05$)

Compared to the methanol content naturally present in coffee brew, the samples that contained aspartame, CA and AS, presented an increase in methanol of 17.8 and

19.3% when assayed at 90 °C and the values of 32.5 and 26.3% for the temperature of 180 °C. These values demonstrate the effect of temperature on aspartame degradation.

Regarding the effect of pH on the production of methanol in an aqueous solution of aspartame subjected to different temperatures (Table 2), alkaline pH had a greater effect on methanol production. In this case, the temperature also influenced the production of methanol, with a larger amount of methanol being produced at a higher temperature. These data were expected since it has been reported that aspartame is more stable at acidic pH (BELL & LABUZA, 1994). The observed degradation of aspartame in aqueous solutions with increasing temperatures can be more drastic when the pH is high. Other investigators have observed that aspartame is decomposed when the solution is heated in an acidified and dry medium (GRAVES & LUO, 1987).

Table 2. Influence of pH on the production of methanol (μg ml^{-1}) from an aqueous solution of aspartame

pH	90 °C	180 °C
4.0	$23.97\pm1.13^{a,1}$	$39.76\pm6.74^{b,1}$
5.0 (Coffee)	$29.16\pm2.12^{a,1}$	$38.28\pm3.11^{a,1}$
8.5	$374.19\pm15.40^{a,2}$	$426.07\pm37.51^{b,2}$

Different letters indicate statistical differences in the lines and different numbers indicate statistical differences in the columns ($P<0.05$)

3. Conclusion

This research investigated the methanol released from aspartame when it is used to sweeten coffee brew. Our data show a protective effect of pH of coffee on degradation of aspartame. This is an important fact mainly for specific populations like diabetics that consume sweeteners like aspartame.

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