

# Ad Libitum Mixing in a Taste Memory Task: Methodological Issues

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#### **Abstract**

Ad libitum mixing, an application of the method of adjustment in food research, was investigated and evaluated for the purpose of taste memory research. The difference between ascending and descending runs in mixing was studied using a wide range of prefill concentrations lower and higher than standard. The effect of training was studied by comparing subjects with two or 10 replications in the first session where a standard was present as a reference. Results showing higher reproduced concentrations after a 25 h time interval than those produced immediately are consistent with earlier results from within-subject designs. Thus, the difference in recall performance did not depend on the design of the study. No difference between ascending and descending runs in the mixing was observed, thus the prefill concentrations did not affect the reproduction of a given standard. There was no significant difference between produced concentrations after two and 10 replications, although a non-significant trend towards improved performance following 10 replications was observed after the 25 h time interval.

## Introduction

The method of adjustment, also known as the method of average error, is one of the classical psychophysical methods of measuring thresholds developed by Fechner in the nineteenth century (Gescheider, 1985). A subject receives a standard stimulus (SS) and matches the intensity of a comparison stimulus (CS) to the SS. The matches should be distributed around the SS. Their mean is the point of subjective equality (PSE), and the standard deviation is interpreted as the just noticeable difference (JND) (Pangborn, 1984; Gescheider, 1985).

In food science, the method of adjustment has been used to determine ad libitum preferences, in which the subjects' task has been to mix salty (Mattes and Lawless, 1985; Blais et al., 1986; Pangborn and Braddock, 1989; Tuorila et al., 1990), fatty (Pangborn et al., 1985) or sweet (Mattes and Lawless, 1985) liquids according to their own preference. Woskow (1967) used ad libitum mixing to evaluate the subjects' sensitivity by asking them to duplicate the standard 20 times by mixing low and high concentrations of taste substance. Ad libitum mixing can also be used to investigate how some added tastant changes the PSE (Woskow, 1967; Pangborn, 1984).

A within-subject design, in which the same subjects have participated in each mixing session at various time intervals, has been used in taste memory research using *ad libitum* mixing (Tuorila *et al.*, 1996; Vanne *et al.*, 1998). In these studies, the subjects' task was to taste the SS and produce an

identical concentration immediately after tasting and again after different time intervals, by mixing low and high concentrations of taste substance into the CS. The reproduced absolute concentrations were higher after all time intervals than in the presence of the standard (Vanne et al., 1998). In the same data, using the relative differences from the standard, the immediately produced concentrations differed only from concentrations produced after 125 h. The results show that the increase in recalled taste intensities is quite slow (Vanne et al., 1998). The between-subject design, in which different subjects work with different concentrations and time intervals, has also been used in taste memory studies by Barker and Weaver (1983). Their subjects compared the memory image of an earlier presented 15% sucrose concentration after one of 1 min, 5 min, 15 min or 72 h time intervals with one of 5, 10, 15 or 20% sucrose concentration. The subjects' task was to report whether the presented concentration was less sweet, equally sweet or sweeter than the standard. Subjects rated the 15% sucrose concentration sweeter than the 15% sucrose standard after all time intervals.

The unsolved question on the possible difference between within- and between-subjects designs needs attention. It is possible that in the within-subject design, the subjects reproduce a given standard using not the image of the standard but the image of their earlier mixture as a reference. If both designs lead to similar outcome, the

within-subject design with smaller error variance gives more information with less subjects.

Two kinds of results have been reported on the role of prefills in ad libitum mixing. Investigating ad libitum preferences, Mattes and Lawless (1985) found the adjusted concentration that was produced from a higher than standard prefill to be higher and from a lower than standard prefill to be lower than the standard. However, the subjects were able to distinguish between the two adjusted samples, which shows that the adjustment error did not depend on perceptual insensitivity. In the taste intensity memory study by Tuorila et al. (1996), prefills (range 0-20%) had no significant effect on the final concentrations. The major difference between these two designs is that Mattes and Lawless (1985) asked subjects to produce the most preferred concentration, while Tuorila et al. (1996) asked subjects to reproduce the previously presented standard. As Mattes and Lawless (1985) noted, the hedonic JND does not necessarily correspond to the JND in intensity. They suggest that the effect of prefills is similar to the error of anticipation, in which an early change in response is due to the subject's imagination that something will soon occur. Thus, the difference between tasks can lead to the different effect of the prefills. Another possible explanation for the difference is the number and the distribution of the prefills. Mattes and Lawless (1985) used only two prefills (low and high), while Tuorila et al. (1996) had more prefills above the standard than below it. A systematic variation of the prefills from both sides of the standard will give more specific information about the role of prefills in a taste memory task.

The role of training in taste memory research has not received much attention. However, in many sensory tests, and also in the selection of a panel, there is an implicit assumption that subjects or panel members will memorize properties of the product that is under examination. Yet the number of training sessions needed is an open question. Can the assessors reproduce a previously presented sweet taste intensity more accurately if the standard concentration is presented to the assessors several times rather than only a couple of times? In earlier research, subjects participating in six training sessions during 8 days produced concentrations

closer to a standard after different time intervals than subjects with minimal training (Tuorila et al., 1996). In some studies, all subjects were untrained (Barker and Weaver, 1983; Vanne et al., 1998). In studies working with mnemophysical power functions (Algom and Marks, 1989; Algom and Cain, 1991; Algom et al., 1993; Stevenson and Prescott, 1997), the subjects were trained before the experiment until they achieved a specific learning criterion.

The aim of this study was to investigate methodological questions in ad libitum mixing used in memory studies. The previous use of the within-subject design raised the question regarding the subjects' ability to reproduce a given standard using the image of the standard as a reference. Possibly, they use the image of their earlier mixture. In the present study, we used a between-subjects design with the same kind of procedure and a similar range of time intervals as in the earlier taste memory studies. The sucrose concentration to be recalled was presented in water. A large range of prefills was used to test the effects of prefills in ad libitum mixing in a situation in which the subjects' task was to reproduce a given standard. The role of training the mixing procedure in recalling taste intensities was investigated by varying the number of replications (2 versus 10) in the first session, in which the standard stimulus was available during ad libitum mixing.

#### Materials and methods

The subjects were 96 volunteers, 63 females and 33 males, students and staff members at the University of Helsinki. Their ages ranged from 18 to 50 years. Some of them had had previous experience in sensory analysis, but none of them had participated in a taste memory experiment before. Subjects were told that the aim of the study was to investigate memory for tastes. There were six groups and two training groups. The number of subjects participating in each group and the tasks of each group are shown in Table 1.

#### Stimuli

Sucrose was used as standard stimuli at 4.1 and 14.6% concentrations (w/w), respectively, in tap water. The

 Table 1
 Subjects, concentrations and number of replications used in each group

Group	n	F	М	Mean age	Age range	Standard % sucrose	Replic. session I	Time interval (h)	Replic. session II
1	11	7	4	23	19–36	4.1	2	1	10
2	12	8	4	26	22-48	4.1	2	25	10
3	13	7	6	27	19–50	4.1	2	125	10
4	13	9	4	25	19~36	14.6	2	1	10
5	13	8	5	28	20-43	14.6	2	25	10
6	11	8	3	26	19-30	14.6	2	125	10
7	13	9	4	23	20-42	4.1	10	25	10
8	10	8	2	23	20–28	14.6	10	25	10

standard stimuli and the low (0%) and high (33.9%) concentrations (w/w) of sucrose for mixing, 60 ml each, were presented in 100 ml beakers. The subjects were advised to ask for more solutions if they needed them. Ten different prefill concentrations of sucrose were used with both standard stimuli. The prefills, based on the Weber ratio (Lundgren et al., 1976), were expected to be 2, 4, 6, 8 and 10, just noticeable differences lower and higher than the intensity evoked by the standard concentration. The concentrations of the standards are presented in Table 1. The concentrations of prefills [range 2.0-8.2% (w/w) for the lower standard and 7.5-26.4% (w/w) for the higher standard] are shown in Figure 2. The prefills (20 ml each) were presented in 110 ml plastic cups.

The CS prepared by the subjects were kept frozen at -20°C until they were analysed refractometrically at 20°C. The transformation from refractive indices to sucrose concentrations (w/w) was carried out using reference tables (AOAC, 1990).

#### **Procedure**

In the first session, subjects received one of the two standard stimuli (see Table 1), low and high concentrations of sucrose for mixing, one training cup with prefill (not used in data analysis) and two cups of prefills (one lower and one higher than the standard). These prefills were chosen at random from the available set. The subjects were asked to pay attention to the sweetness intensity of the standard stimulus. First the subjects tasted both the low and high concentrations of sucrose for mixing, and rinsed their mouth with tap water after each tasting. Then they tasted the standard and the prefill in the training cup, and tried to reproduce the intensity of the standard concentration by mixing low and high concentrations of taste substance in the training cup. After training, the subjects mixed the same sweetness intensity twice, once from the low and once from the high prefill. They were instructed to taste the same standard again before each mixing task and were allowed to taste the standard as often as they needed. After each mixing, the subjects had a 1 min break which began by rinsing the mouth with tap water for 15 s (O'Mahony, 1986). The second session was held 1, 25 or 125 h after the first session. In the second session, the subjects received 10 cups with randomized prefills (five lower and five higher than the standard) and were asked to manipulate the prefills so that they were identical to the taste of the previously tasted standard. After preparing each mixture, the subjects put the lid on the cup, thus retasting a ready mix was not possible. Two of the groups (group 7 for low and group 8 for high concentration) mixed the standard 10 times in both the first and second sessions. The second session for training groups was held after 25 h.

The subjects worked in a sensory laboratory with partitioned booths for tasting and spitting. At the beginning of both sessions, the subjects received detailed instructions individually and they were also allowed to ask for further details if needed.

## Data analysis

All the data analyses were carried out using relative differences of mixed concentrations from standard stimuli [(mixed concentration – standard)/standard]. The change in relative differences between the two sessions was analysed by multivariate ANOVA using standard concentration (4.1% versus 14.6%) and time interval between sessions (1, 25 and 125 h) as sources of variance. A paired t-test was used to compare the relative difference from the standard stimuli between the first and second session.

The training effect was measured by comparing groups with 10 replications to groups with two replications with the same time interval (group 2 versus group 7 and group 5 versus group 8). This was done by multivariate ANOVA with standard concentration (4.1% versus 14.6%) and number of replications in the first session (2 versus 10) as sources of variance.

In the groups with 10 replications, the effect of prefills was measured by repeated measures ANOVA using session (2) and prefills (10) as factors and standard concentration (4.1% versus 14.6%) as a between-subjects variable.

The difference of each group from zero was measured by a one-sample t-test.

### Results

The difference between the two sessions was greater with low than with high standard concentrations [main effect for concentration F(1,67) = 7.55, P = 0.008] (Figure 1). Subjects produced higher sucrose concentrations after different time intervals than in the first session [main effect for time F(2,67) = 3.36, P = 0.041]. The significant interaction between standard concentration and time interval [F(2,67)]4.88, P = 0.011] shows that the effect of time can be observed mostly from concentrations produced with the low standard as the target, while the concentrations produced with the high standard concentration as the target were more accurate. This was confirmed by significantly higher concentrations after 25 h with the low compared with the high standard concentration [t(11) = 3.44, P = 0.006]. No other differences between time intervals were observed.

There were no differences between concentrations mixed from prefills lower and higher than the standard [t(72)] = 0.74, P = 0.46] (Figure 2), nor was any effect of prefills observed in the groups with 10 replications [F(9,189) = 0.76,P = 0.65].

All groups except one (two replications, 14.6% standard, 25 h time interval) (Figure 1b) produced a higher concentration than the standard in the first session (P <

Increasing the number of replications in the first session from two to 10 did not affect the concentrations produced

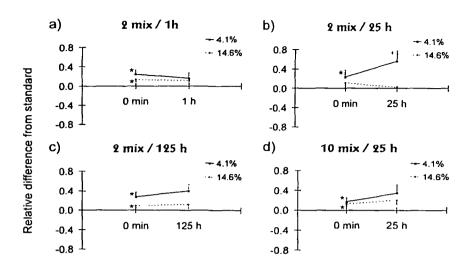


Figure 1 Relative differences from the standard stimuli (+ SEM) in the first session (0 min) with two replications and after 1 h (a), 25 h (b) and 125 h (c) time intervals as well as with 10 replications in the first session and after 25 h (d) time interval. (+) = significant difference between relative differences from the standard in the first and second session; (\*) = significant difference from the standard concentration in the first session.

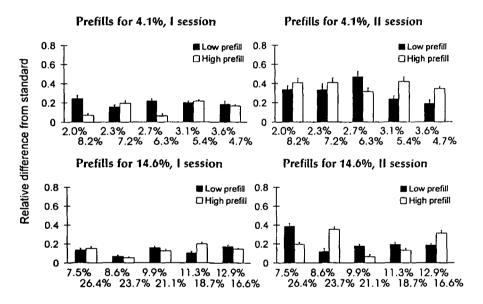


Figure 2 Relative differences from the standard (+ SEM) with different prefills lower and higher than the standard.

[F(1,44) = 0.01, P = 0.91]. The relative differences were again higher with the low standard than with the high standard [F(1,44) = 7.60, P = 0.009]. Although the interaction between standard concentration and training was not significant [F(1,44) = 2.90, P = 0.10], the concentrations produced in the second session tended to be closer to the first session concentrations in groups with 10 replications in the first session (Figure 1).

#### Discussion

The tendency to recall taste intensities as more intense than the standard was observed in this study in a between-subject design. Concentrations produced after 25 h using 4.1% sucrose as a standard stimulus were significantly higher than those produced initially in the presence of standard. Previous taste memory studies, in which ad libitum mixing was used in a within-subject design (Tuorila et al., 1996; Vanne et al., 1998), produced similar results. The higher absolute sucrose concentrations in red currant juice after 24 h, 1 week and 6 weeks (Tuorila et al., 1996) and the higher relative differences from standard after 125 h in water (Vanne et al., 1998) both concur with the higher relative difference from the standard in the present study using 4.1% sucrose after 25 h than in the first session. The lack of effect of 14.6% sucrose can be related to the concentration of the memorized stimulus. In an earlier study, the relative

difference from standard was lower with 16.1% sucrose than with 4.2% sucrose (Vanne et al., 1998). The present study also replicates our earlier results (Tuorila et al., 1996; Vanne et al., 1998) that after a short time interval the concentrations produced are closer to the standard than after longer time intervals. Obviously both designs give similar results and reproducing the standard is not dependent on whether a between- or a within-subject design is used.

The prefills did not affect the outcome. No difference between concentrations produced from prefills higher or lower than the standard was observed. Mattes and Lawless (1985) obtained the opposite result with subjects producing a preferred level of tastant. The difference between the results can possibly be explained by a wider range of acceptable preference concentrations compared to the situation in which subjects are instructed to reproduce a given standard.

Increasing the number of replications in the first session tended to help (even though not significantly) subjects to produce more similar concentrations after 25 h. In earlier research (Tuorila et al., 1996), subjects with no training (only the mixing session) produced higher absolute concentrations than subjects with six training sessions. The difference in results between the earlier study (Tuorila et al., 1996) and the present one can possibly be explained by the larger values of absolute concentrations compared to relative concentrations. However, the training seems to help subjects in a memory task. More research is needed to investigate how much training is needed and for how long, e.g. in training a sensory panel. A tailored training with feedback on individual performance could help the subjects to recall the standard concentration more accurately.

Our results showed a difference between the first and second session at 4.1% concentration, but not at 14.6% concentration. One possible explanation may be the different location on psychophysical function for sucrose. The function is compressive (McBride, 1989; Schifferstein and Frijters, 1990); thus, the relative increase in sweetness decreases when the concentration of sucrose increases. This can have an effect on the relative difference between concentrations. However, the function is almost linear at the concentration level used in this study (McBride, 1989; Schifferstein and Frijters, 1990), so it is quite obvious that the psychophysical function does not explain the differences between relative differences.

All groups except one (two replications, 14.6%, 25 h time interval) (Figure 1b) produced a higher concentration than the standard in the first session. This phenomenon needs more clarification. The role of adaptation in ad libitum mixing has been discussed by Mattes and Lawless (1985). They noted that after tasting a very concentrated sample, the subject's receptors for the particular tastant could become partially adapted and less responsive to the tastant, and because of that the adjusted concentration would be too high. In the present study, the adaptation was prevented by asking subjects to rinse their mouth before tasting the standard stimulus. According to O'Mahony (1986), due to adaptation, a constant taste stimulus will be perceived as decreasing in intensity. However, the standard concentration cannot be considered a constant stimulus in that way because the subjects tasted different concentrations all the time during mixing.

The role of high sucrose concentration for mixing in the ad libitum design must be taken into account when discussing the role of prefills or higher than the standard concentrations in the first session. If a subject working with a prefill lower than the standard adds too much strong sucrose, he or she will exceed the standard and perhaps also go over the next discrimination threshold. Decreasing the reproduced concentration with water is slower and will not result in a return to the other side of the standard. This can result in a situation in which all subjects with all concentrations are actually mixing water to higher than the standard concentration in order to decrease the concentration. In this kind of situation, it is possible that all subjects will stop the mixing when they have reached the last discrimination threshold before the standard. This phenomenon can take place at a low standard concentration, where a small addition of high sucrose concentration can cause a large change in the prefill.

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